Chromosomes, Disease and Gene Mapping

DK Griffin

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- What are chromosomes?
- How do we make chromosomes?
  - Samples
  - G-banding
  - FISH
- How to we analyse chromosomes?
- Chromosomes and disease
  - Numerical abnormalities
  - Structural abnormalities
  - Fertility issues
  - Cancer
- Diagnosis and genetic counselling
  - Referral categories
  - Prenatal diagnosis (why, how, what next?)
- Chromosomes and gene mapping
- Nuclear organisation, development and disease
- Copy number variation (CNV)

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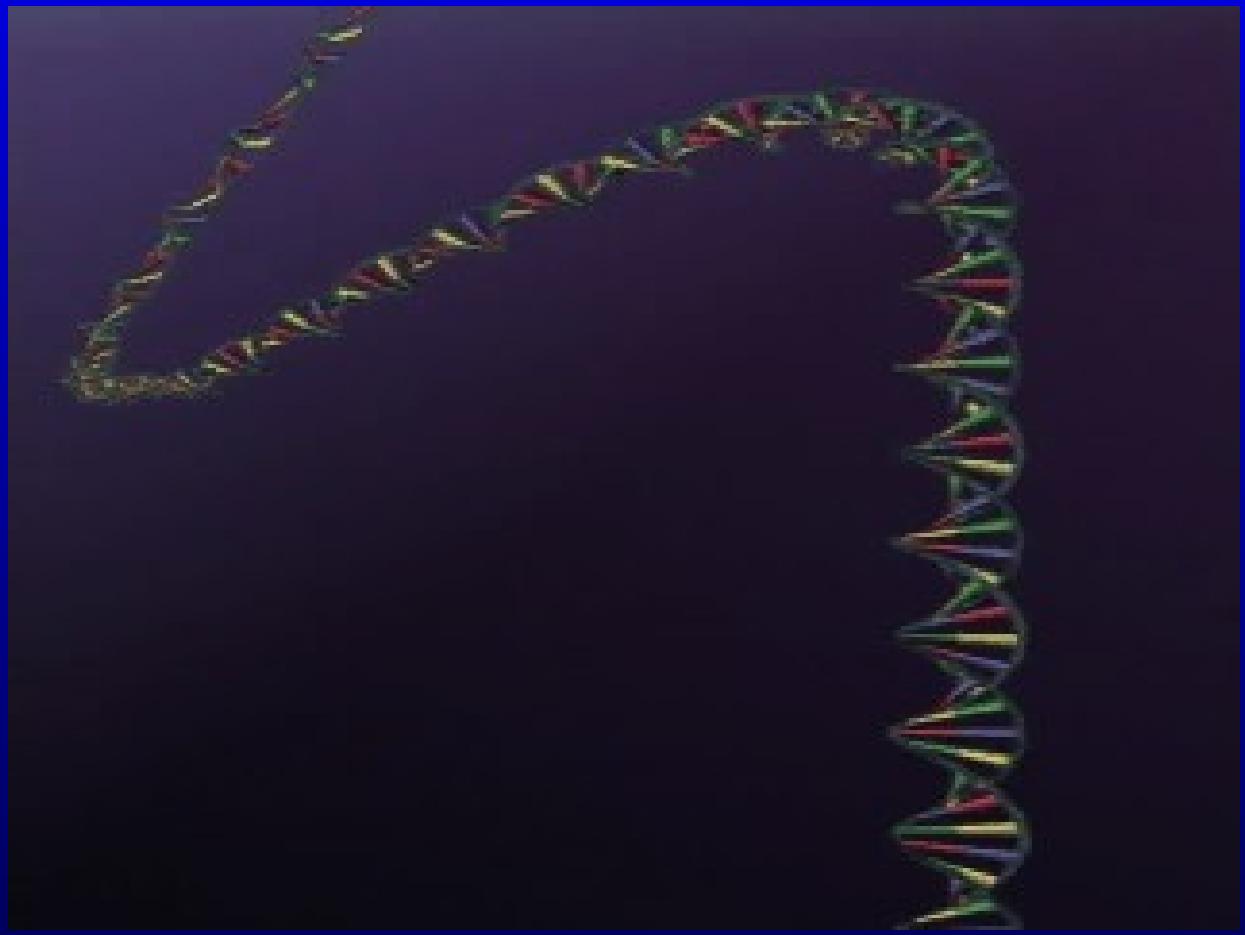
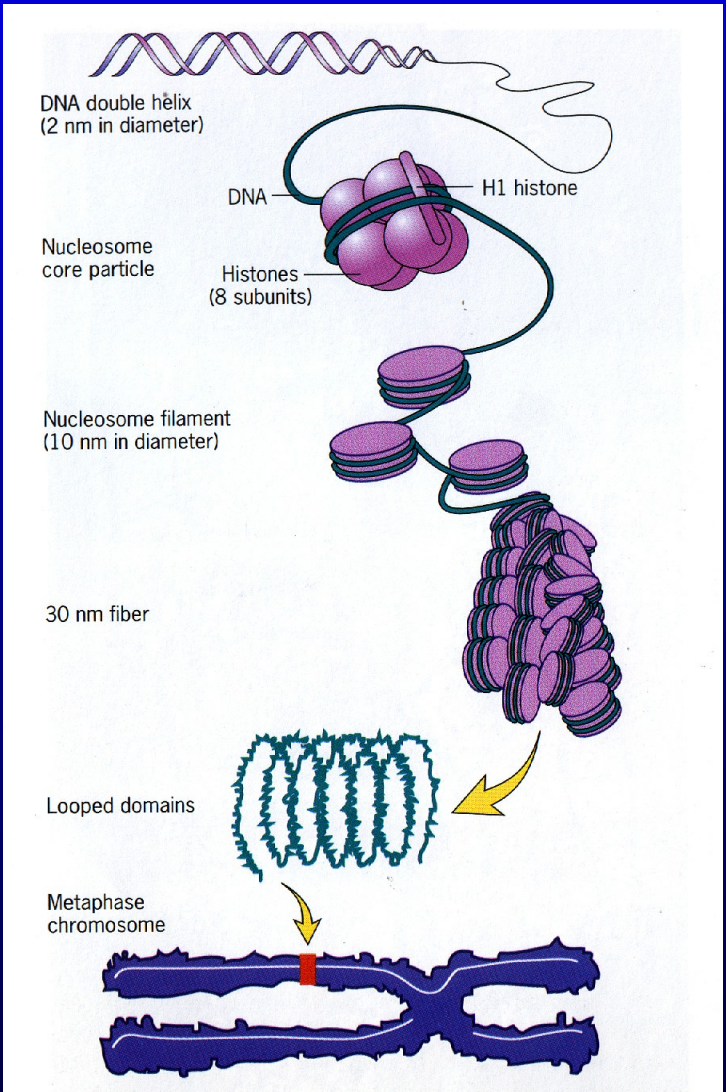
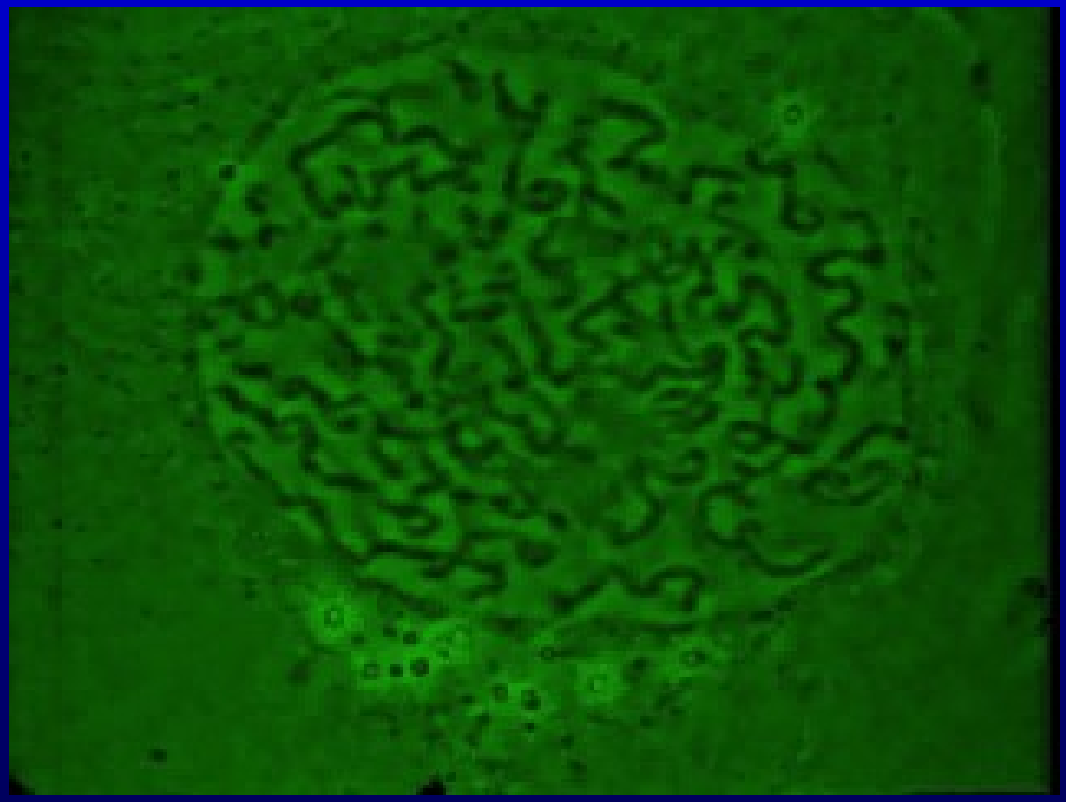


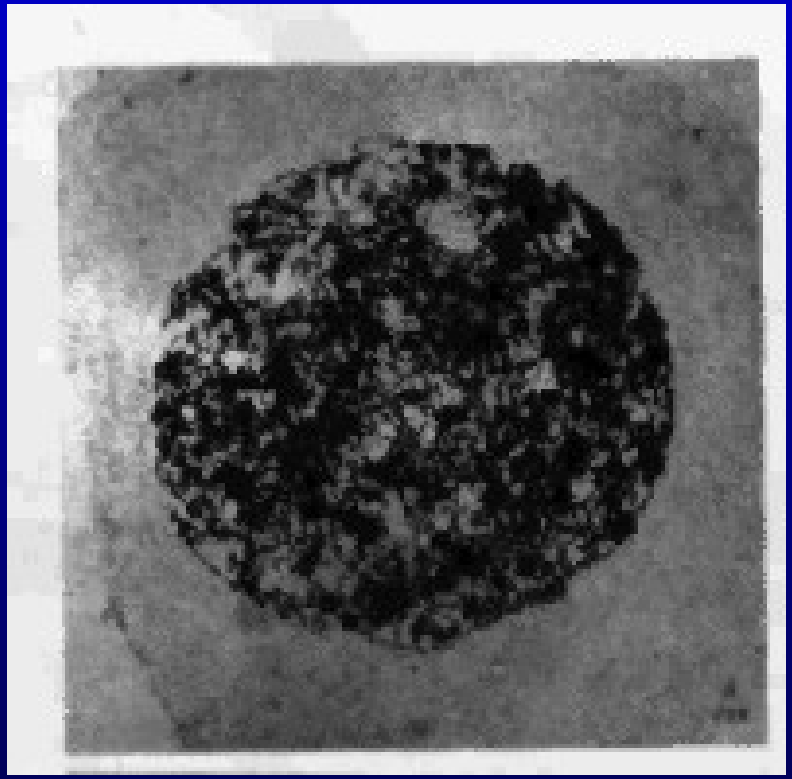
Formation of chromosomes



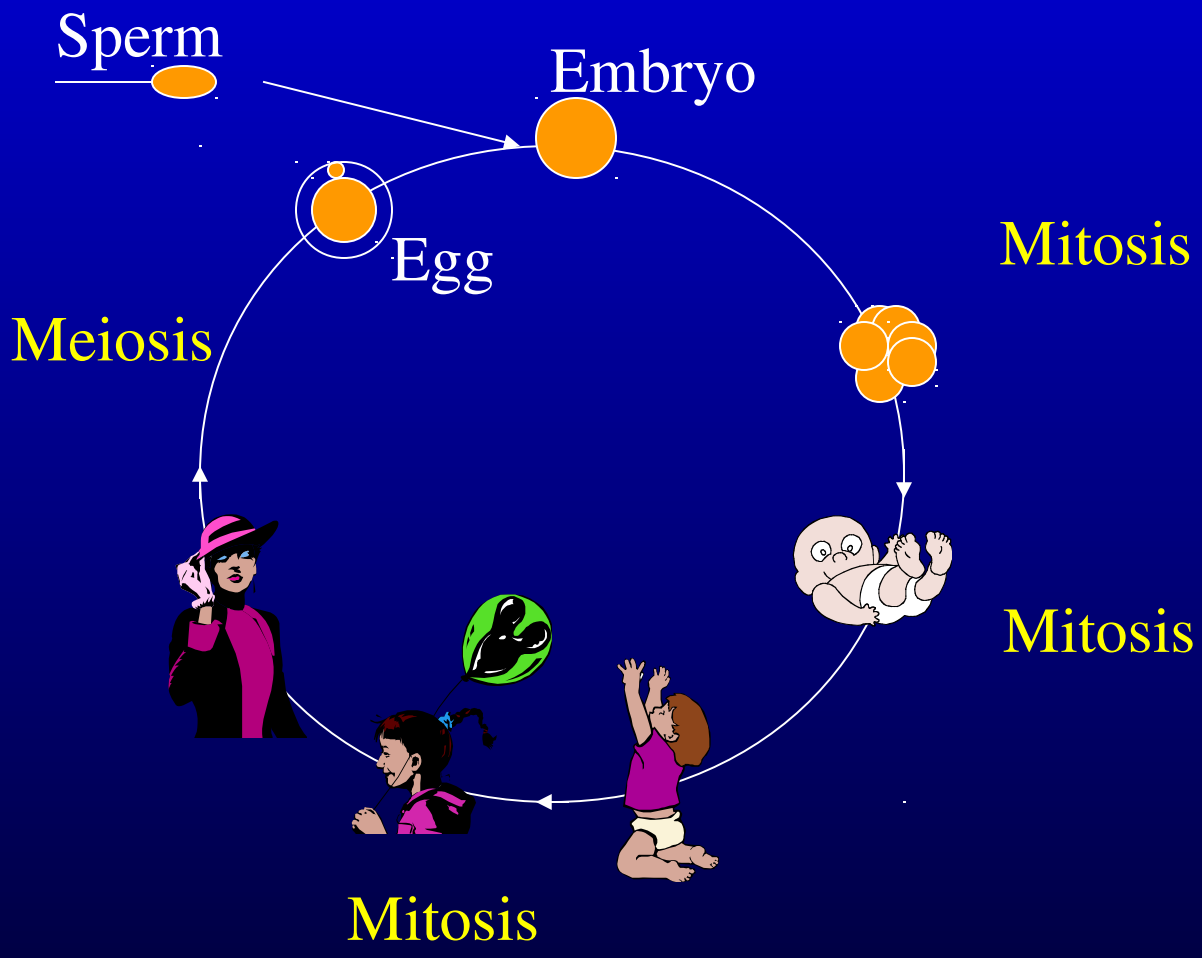
Mitosis



Meiosis



Animal life cycle

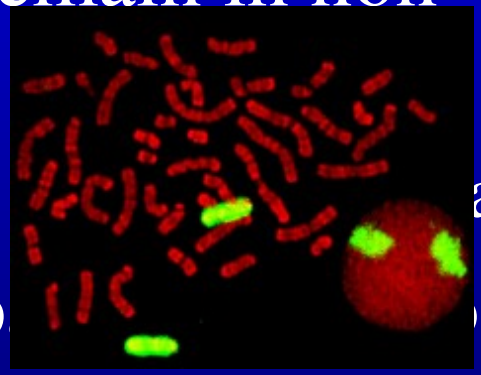


What are chromosomes?

- DNA and protein coils form chromosomes
- Chromosomes important in disease
- Study of chromosomes important for gene mapping
  - Genes always in the same order on the chromosomes
- Genes usually silent when folded into a chromosome
  - But active when in the nucleus

What are chromosomes?

- Chromosomal domains remain in non-dividing nucleus
  - Genes usually active
- Chromosomes occur in p
  arranged -> Karyotype
  - Each species has an individual karyotype
  - Clinical uses (later)



Different chromosomes

Human = 46

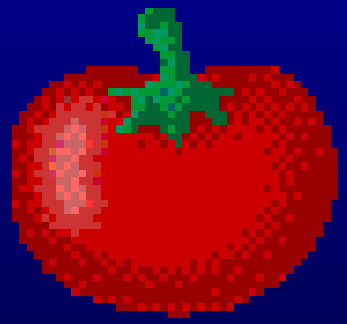
Chicken = 78

Other species

78

64

26



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Preparation of chromosomes: Five principles:

1. The cell must be dividing,
2. The cell must be arrested in a dividing state
3. The cell must be swollen osmotically to spread the chromosomes,
4. The cell must be fixed to a glass slide,
5. The chromosomes must be stained for identification
   - G-banding
   - FISH

Origin of samples

- Blood or tissue (e.g. skin) from individual
  - Post-natal diagnosis
  - Blood culture is the most used tissue for human chromosome analysis
- Amniotic fluid, chorionic villus sample, umbilical cord blood
  - Prenatal diagnosis
- Sperm
  - Fertility studies

Chromosome preparation from blood

- Culture medium
  - Water, balanced salts, buffer pH constant
  - Fetal calf serum – natural growth factors
  - L-glutamine promotes growth
  - Antibiotics/fungicides to promote sterility
  - Aseptic technique
  - Mitogen (Phytohaemaglutinin -PHA)
  - Culture for 72 hours
- Use “colchicine” to arrest in metaphase
- Swell up cells with 75mM KCl
- Use methanol/acetic acid (3:1 ratio) to fix the cells to a glass slide
- Stain

Chromosome preparation (post-natal) – bone marrow

- Bone marrow is actively dividing (needs no mitogen)
  - Leukemia studies
- Culture overnight
- Harvest in same way as blood
  - Suspension culture

Chromosome preparation (post-natal) – solid tissue

- Skin
  - Useful for studies when another cell lineage is required for investigation
  - Adherent culture
- Small biopsy
  - Chop finely in culture medium
  - Cells adhere to plastic culture tube
  - Remove cells with trypsin
  - Harvest in same way as blood

Chromosome preparation (pre-natal) – amniotic fluid

- Amniotic fluid cells (14-16 weeks)
- Most common prenatal sample - insertion of trans-abdominal needle under ultra sound guidance
- Cells shed from fetus
- Long term adherent culture
- Harvest as for skin

Chromosome preparation (pre-natal) - CVS

- Chorionic villus (9-13 weeks)
- Insertion of trans-abdominal needle under US
- Trans-vaginally - aspiration, forceps
- High risk pregnancies
- Placenta
  - Mesenchymal core cells
  - Dissect under microscope with needles to remove maternal cells
- Long term adherent culture, harvest as for skin

Chromosome preparation (pre-natal) - CVS

- Direct preparation from trophoblast
- Quick culture in medium with colcemid
- Harvest within few hours with hypotonic and fixative
- Preliminary result
  - Few short metaphases

Chromosome preparation (pre-natal) – fetal blood

- Fetal blood (16-20 weeks)
- From cord - insertion of trans -abdominal needle under ultra sound guidance
- Suspension culture with heparin
- Harvest as for blood

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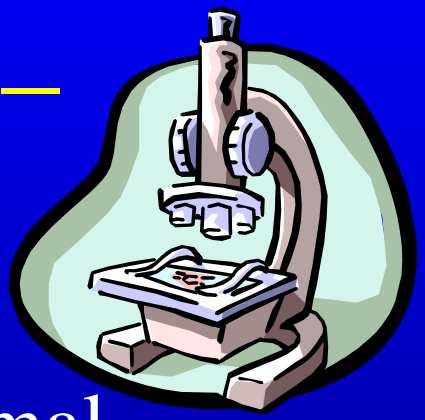
Chromosome staining – G-banding



- Trypsin partially digests chromosomal proteins
- “Giemsa” stain
- Dark and light bands
- Dark AT rich, light GC rich
- Most widely used technique for routine staining

G-Banding

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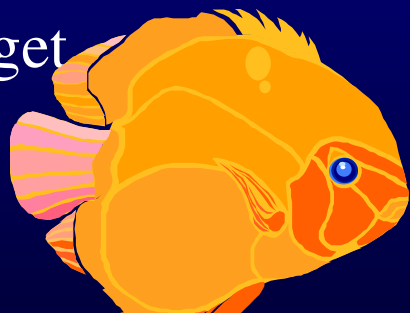
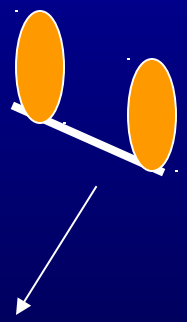
Fluorescent in-situ hybridisation FISH!

- Lighting up chromosomes or chromosome regions at will either on metaphase chromosomes or non-dividing nuclei
- Fluorescent - involving fluorescent dyes
- In-situ - directly on to cell preparations
- Hybridisation - DNA-DNA probe to target
  - Target is the chromosomes
  - Brightness of signal proportional to size of target



Hybridisation

- Probe and target have complementary sequences
- Probe is labelled - enables detection



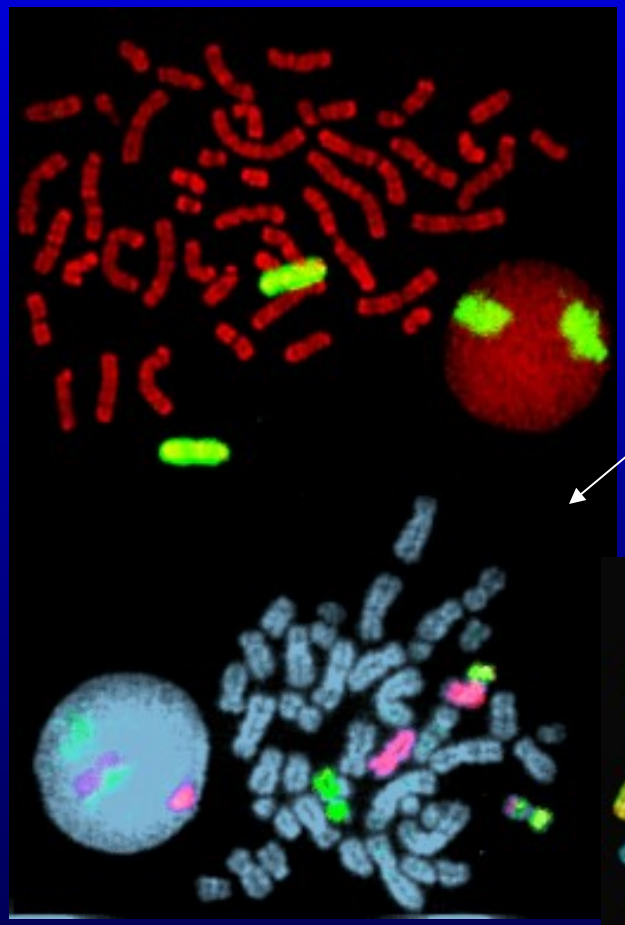
Technical bits

- Chromosomes prepared in a standard way on glass slides
- Probe DNA and target DNA must have strands separated (use of formamide)
- Hybridise probe to target (usually overnight)
- Excess probe washed off (stringency)
- Probe label detected directly or indirectly
- Chromosomes must be stained fluorescently in a different colour

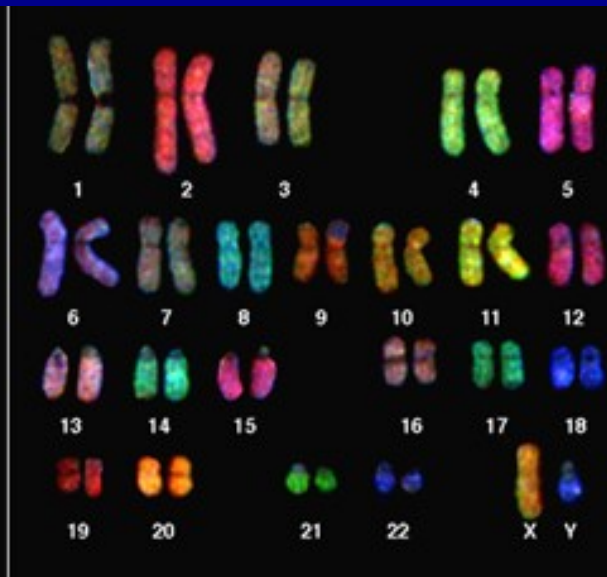
Applications of FISH

- Chromosome painting
- Multicolour chromosome banding
- Gene mapping
- Counting chromosomes in nuclei
- Nuclear organisation

Chromosome painting



- One colour
- Two colours
- 24 colours

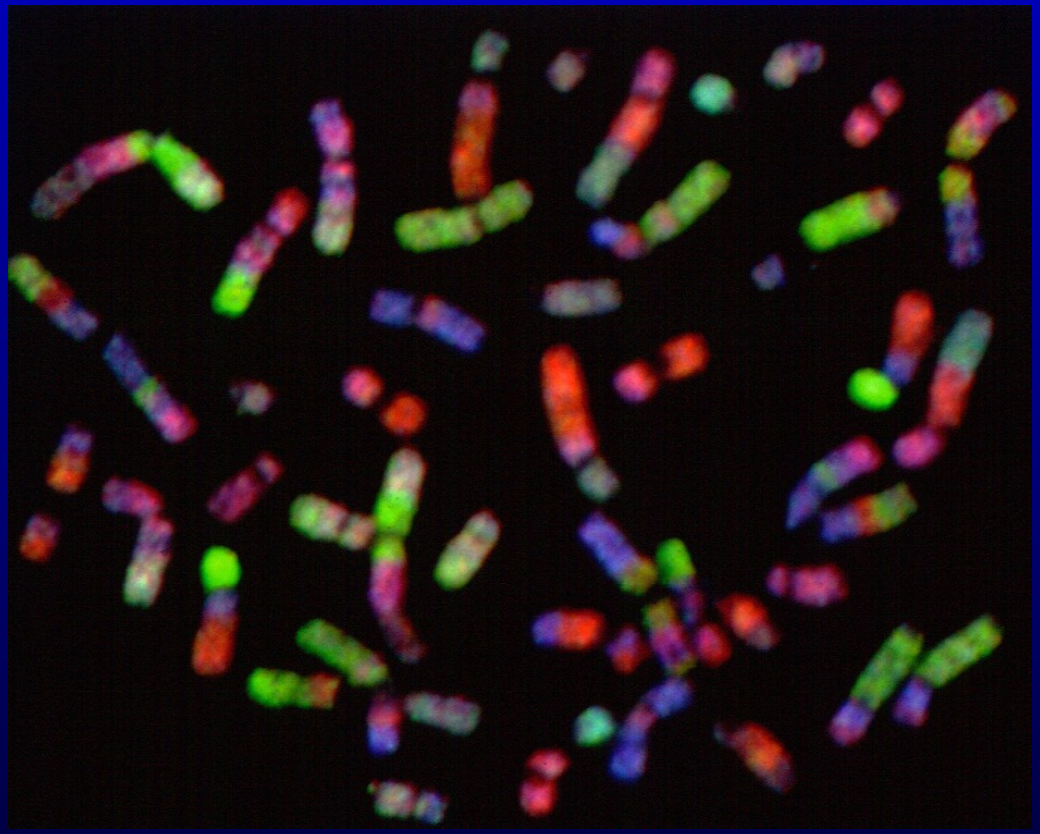


Mixing colours

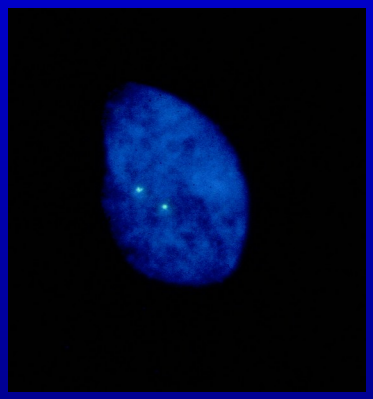
- 2 dyes can give 3 colours (more?)
- 3 dyes can give 7 colours
- 4 dyes can give 15 colours
- 5 dyes can give 31 colours
- What is the formula?
- $2^n - 1$

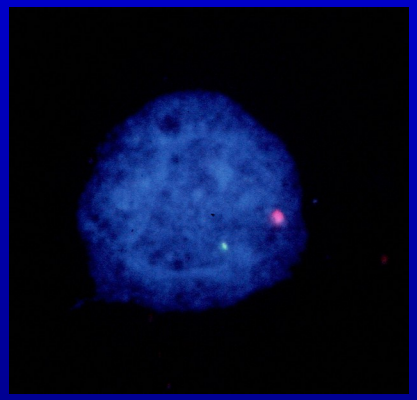
Multicolour chromosome banding

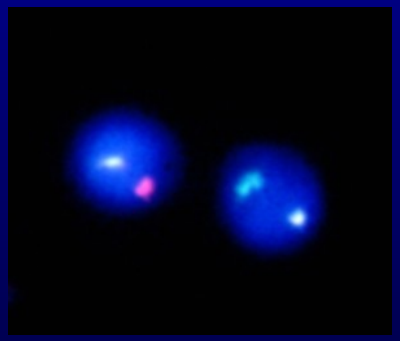
- Alternative to classical banding

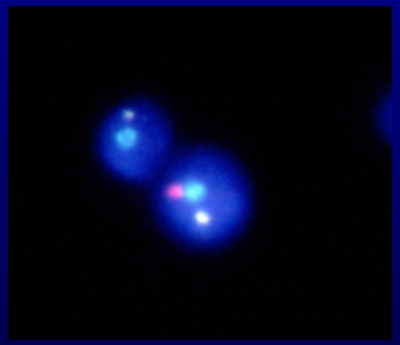


Counting chromosomes in non-dividing nuclei









- Sex chromosomes in single cells from embryos
- Sex chromosomes in sperm
- Also cancer studies

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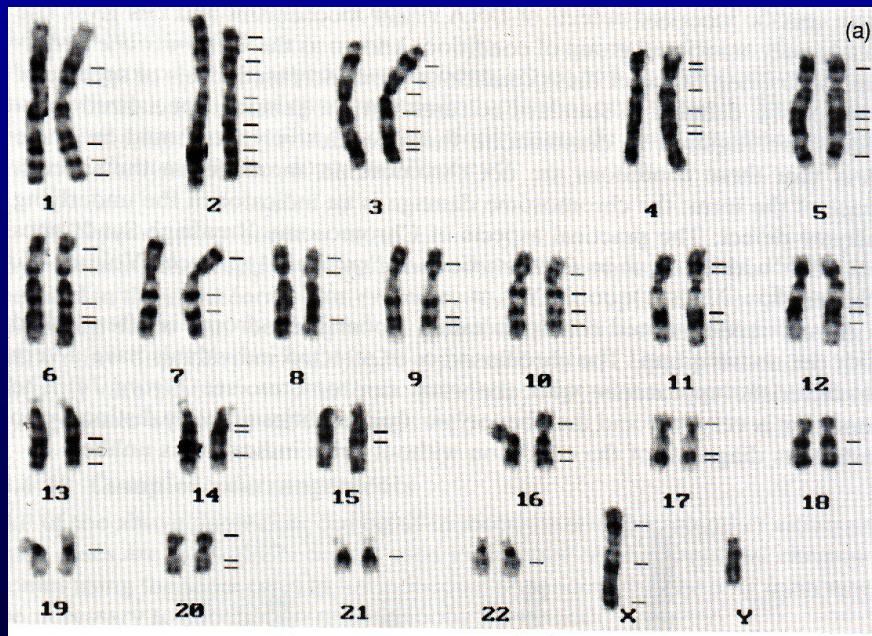
In humans
- 46 chromosomes
- All “easy” to distinguish when G-banded
- KARYOTYPE



How do we get from this?

To this?

Why?

- Deviations from the norm can lead to serious clinical consequences
  - Disease studies
- Gene mapping
  - It is, in effect, a low resolution map of the genome

Karyotyping

- Take a photograph of G-banded chromosomes or capture an image onto computer
- Separate chromosomes by cutting around edges
- Pair them up
- Spot an abnormality or map a gene

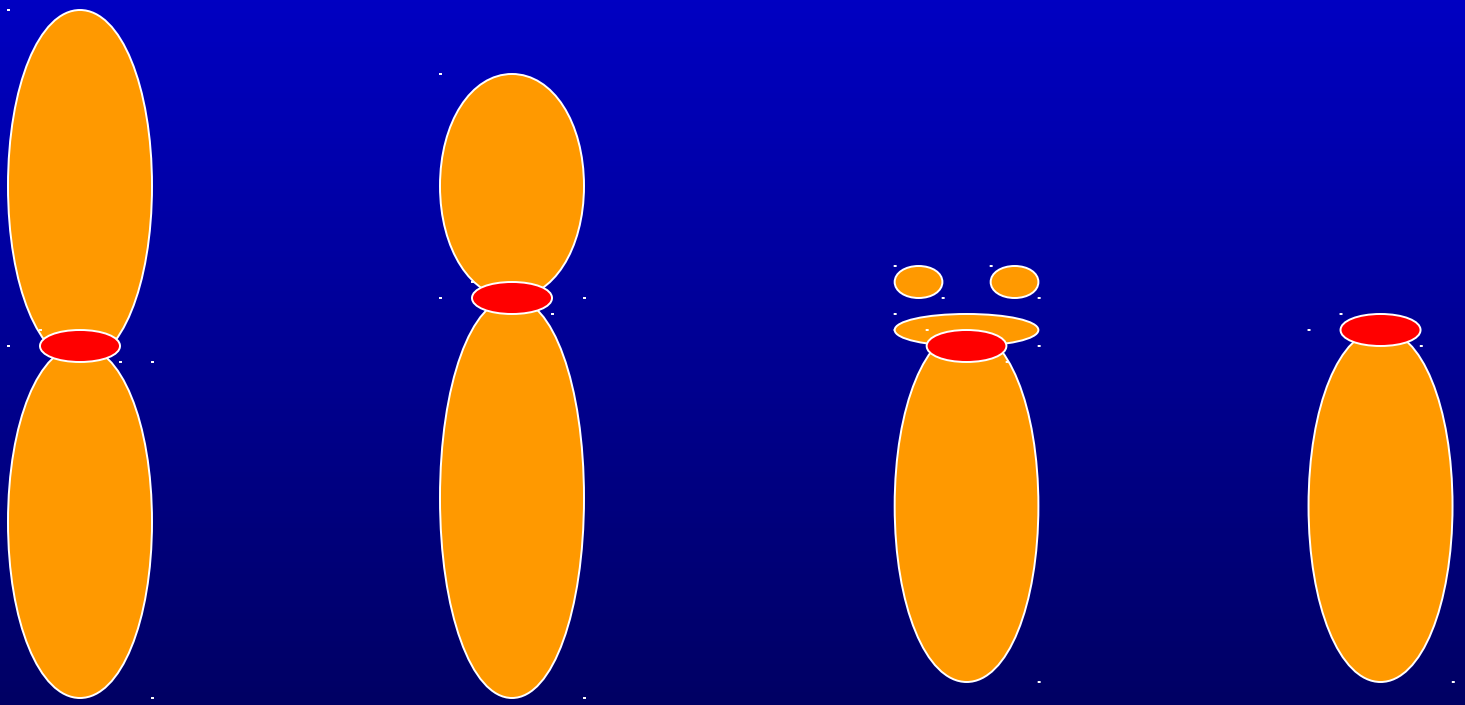
Karyotyping - Golden rules

- Count them
- Arrange them according to size
- Put them in their groups
- Look at the banding

Karyotyping

- Chromosomes are numbered roughly according to size and centromere position
- Human chromosomes are numbered 1-22 (plus X and Y), and subdivided into groups A-G
- Chromosomes can be metacentric, submetacentric or acrocentric

Analysing chromosomes

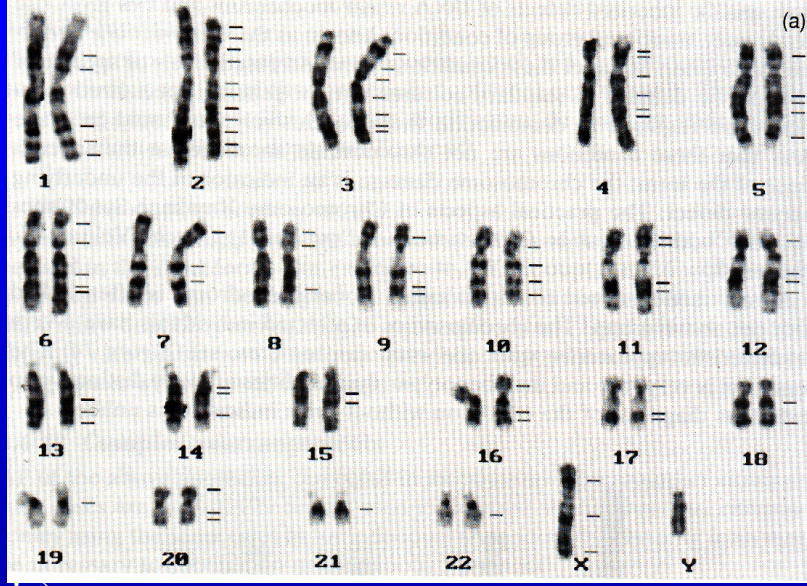


Metacentric Sub-metacentric Acrocentric Telocentric

Analysing chromosomes

- Each chromosome has a characteristic banding pattern
- Some are easier to distinguish than others
- The longer the chromosomes are, the more bands you will see
- Short arm = p-arm
- Long arm = q-arm

Karyotyping



- Group A (chrs. 1-3)
  - Large metacentric chromosomes (2 is submetacentric)
- Group B (chrs. 4-5)
  - Large submetacentric chromosomes, difficult to tell apart
- Group C (6-12 plus the X)
  - Medium sized submetacentric, difficult to tell apart
- Group D (13-15)
  - Medium acrocentric chromosomes with satellites
- Group E (16-18)
  - 16 is metacentric, 17 & 18 are submetacentric, short
- Group F (19-20)
  - Short metacentric
- Group G (21-22 + Y)
  - Sort acrocentric, 21 & 22 have satellites, Y does not.

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Classification of Genetic Diseases

- Autosomal Dominant and Recessive
- Sex linked
  - Usually recessive
- Chromosomal
- Complex
  - Imprinting, Triplet repeat, Mitochondrial
- Multifactorial
  - Most diseases including cancer

Chromosomal disorders

- Numerical abnormalities
  - Aneuploidy (extra or missing chromosomes)
    - E.g. trisomy 21 - Down Syndrome
  - Polyploidy (extra set of chromosomes)
    - Usually results in spontaneous abortions
- Structural abnormalities
  - Deletions, Duplications, Insertions, Unbalanced translocations
    - Usually severe clinical features
  - Balanced translocations, inversions, Y chromosome deletions
    - Mild symptoms but can lead to infertility

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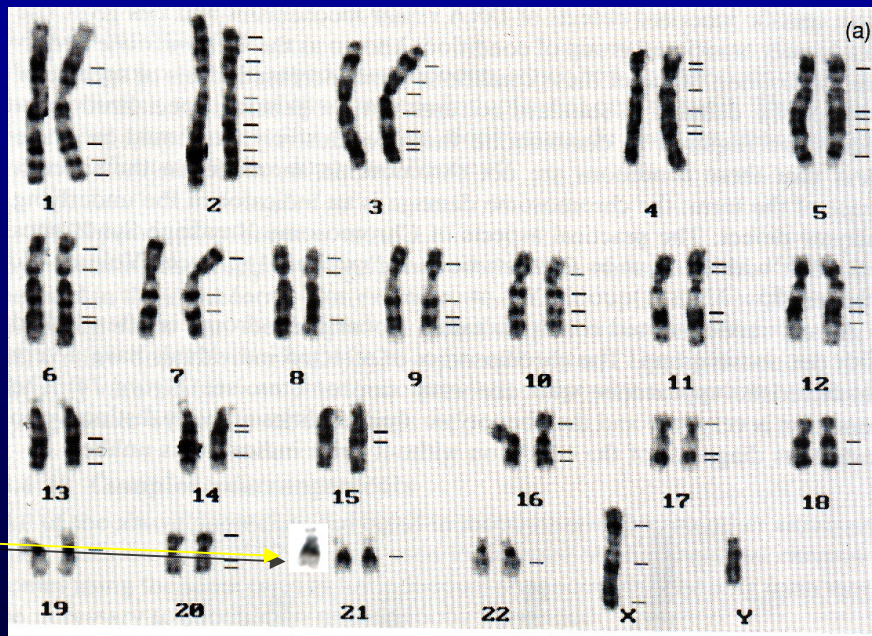
Numerical abnormalities - aneuploidy

- Trisomy (one extra chromosome (47))
  - Trisomies 21, 18 & 13 plus sex chromosomes seen in livebirths
  - Trisomies 21, 18 & 13, 9 and 22 plus sex chromosomes seen in stillbirths
  - Most others seen among spontaneous abortions
- Monosomy (one missing (45))
  - Monosomy X only seen in livebirths
    - Though common in spontaneous abortions
  - Others abort too early to be clinically recognised



Down Syndrome

Down Syndrome - Trisomy 21

- The most common form of aneuploidy in live births (1 in 750)

Trisomy 13 - Patau syndrome

Sex chromosome aneuploidies

XO Turner syndrome

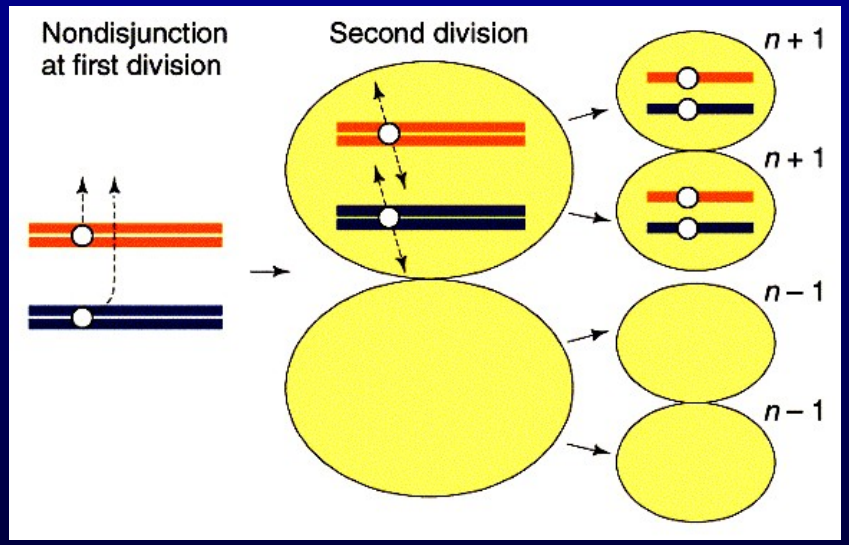
All Infertile

XXY - Klinefelter syndrome

XYY syndrome

Aneuploidy arises by non-disjunction

- Failure of chromosomes to disjoin properly
- Cause of Down syndrome, pregnancy loss
- Some conceptuses have a mixture of normal and abnormal cells (mosaicism)



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Triploidy

- Three sets of chromosomes instead of two (69)
  - Rarely seen in liveborns, common cause of spontaneous abortion
  - Two extra sets (92 chromosomes) -> tetraploidy

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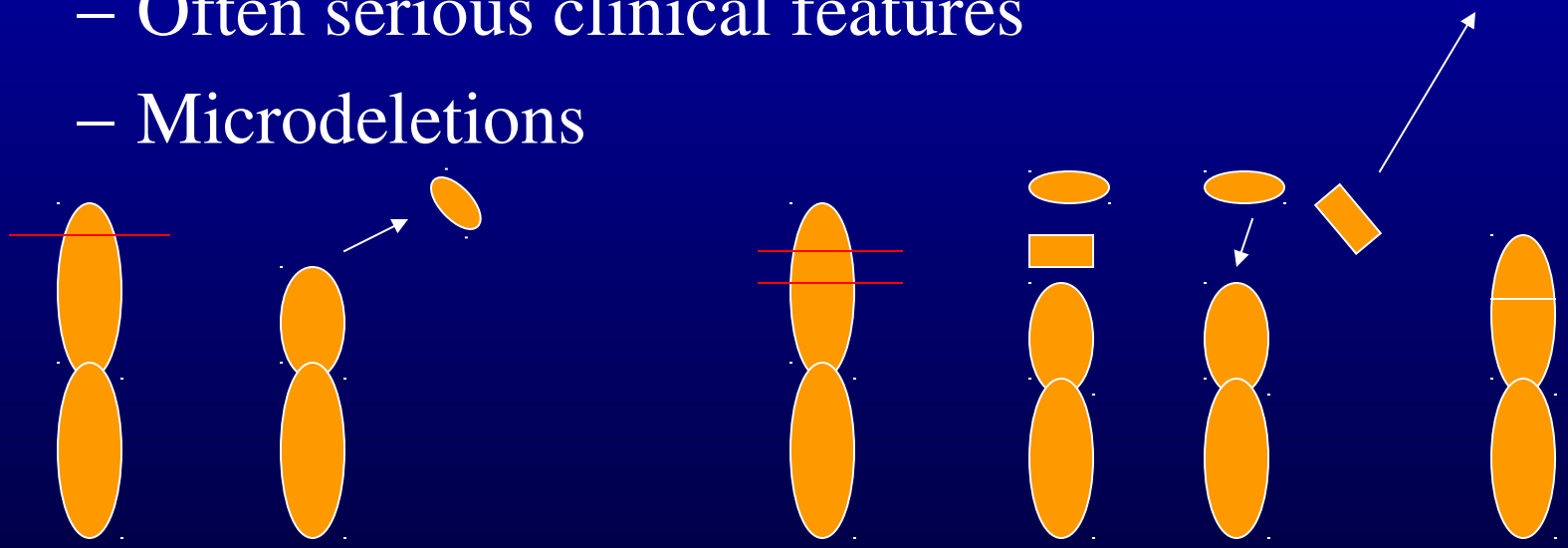
Structural abnormalities

- Deletions
  - Terminal requires one breakpoint
  - Interstitial requires 2
  - Often serious clinical features
  - Microdeletions



Terminal deletion

Interstitial deletion

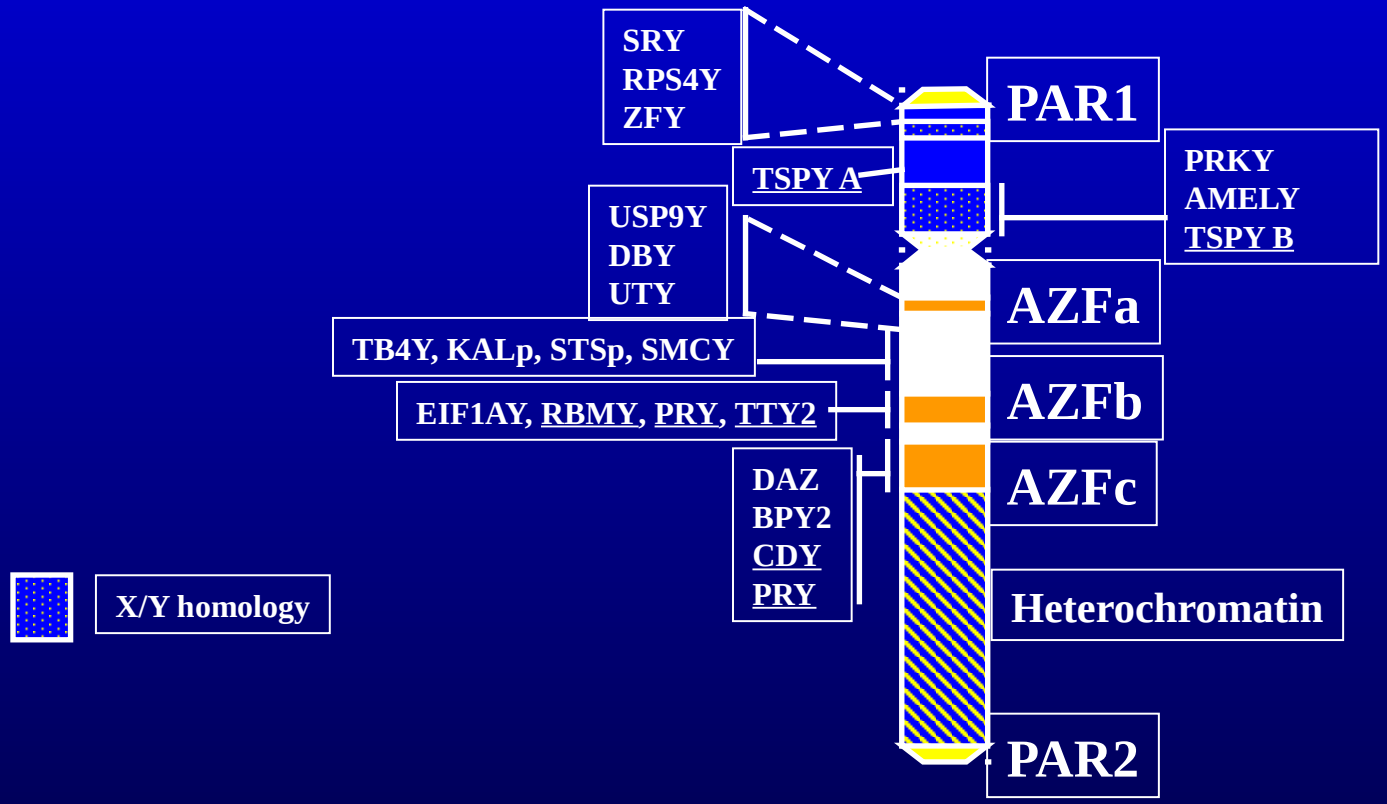
Deletions

- Involve loss of DNA
- Usually severe clinical features
  - Depends on:
  - How much of the genome is lost
  - What part of the genome is lost
  - Whether the Y chromosome is involved

Y chromosome deletions and male infertility

- Very few genes and lots of "junk DNA"
- Evolved from a fully functional chromosome
- Genes involved in spermatogenesis (and hence male fertility) have been retained
- Deletions are common leading to infertility but because there are very few genes on the Y, clinical features are not severe

Human Y Chromosome



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Duplications and Insertions

- Both involve extra pieces of chromosome added and lead to severe clinical features
- Duplication - Extra piece copied and put next to original
- Insertion - Extra piece inserted from another chromosome
- Both result in severe clinical features because of extra DNA

Child with duplication

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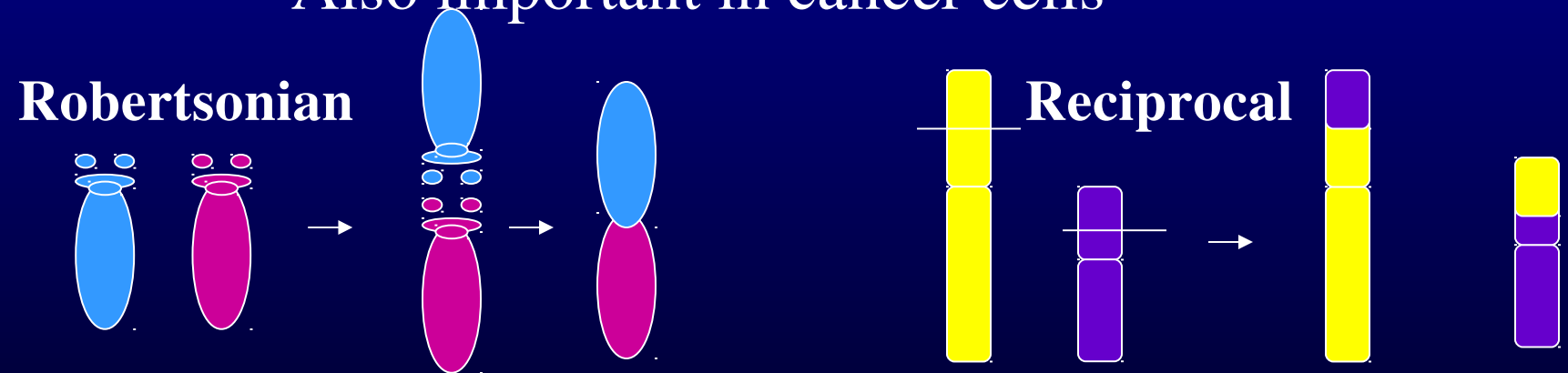
Translocations

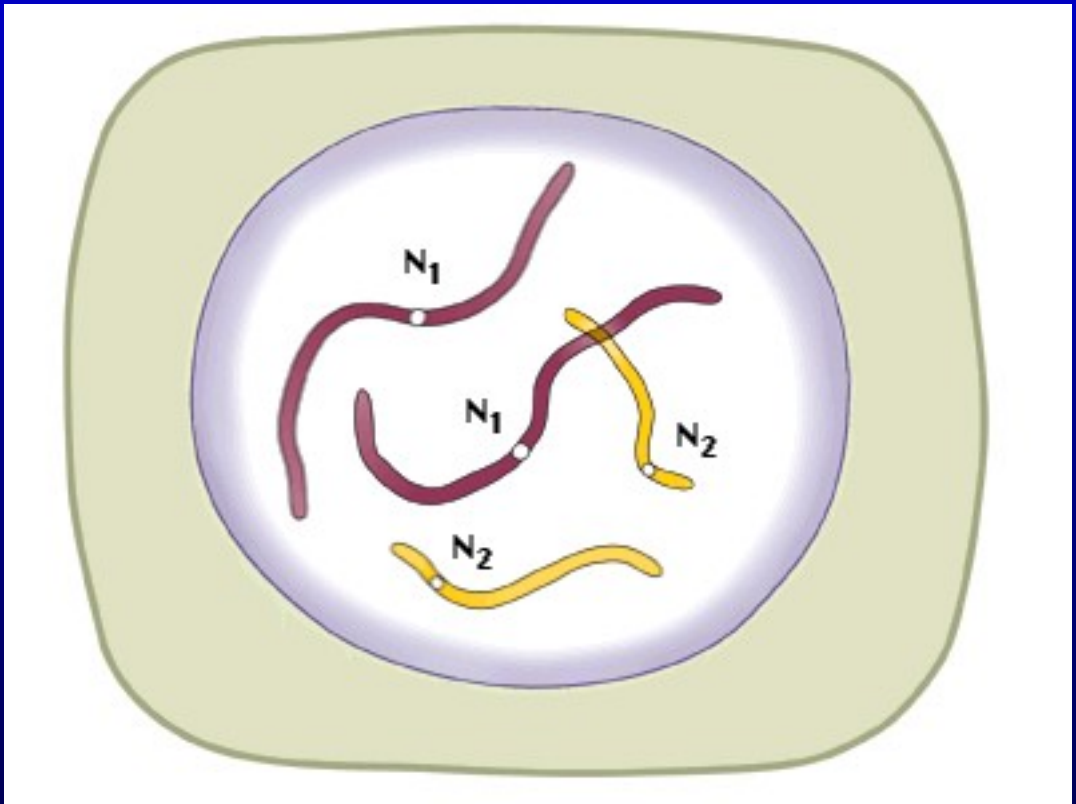
- Unbalanced
  - Often offspring of balanced translocations
    - Loss or gain of material leads to partial trisomy or monosomy
    - Severe clinical abnormalities
- Balanced
  - No net loss or gain of genetic material
    - Usually no phenotypic effect unless gene disrupted by breakpoint, risk to offspring
    - Infertility

Balanced translocations

- Robertsonian translocation
  - End to end fusion of acrocentric chromosomes
- Reciprocal translocation
  - Breaks in two chromosomes
  - Fusion of one to the other and vice versa
  - Also important in cancer cells

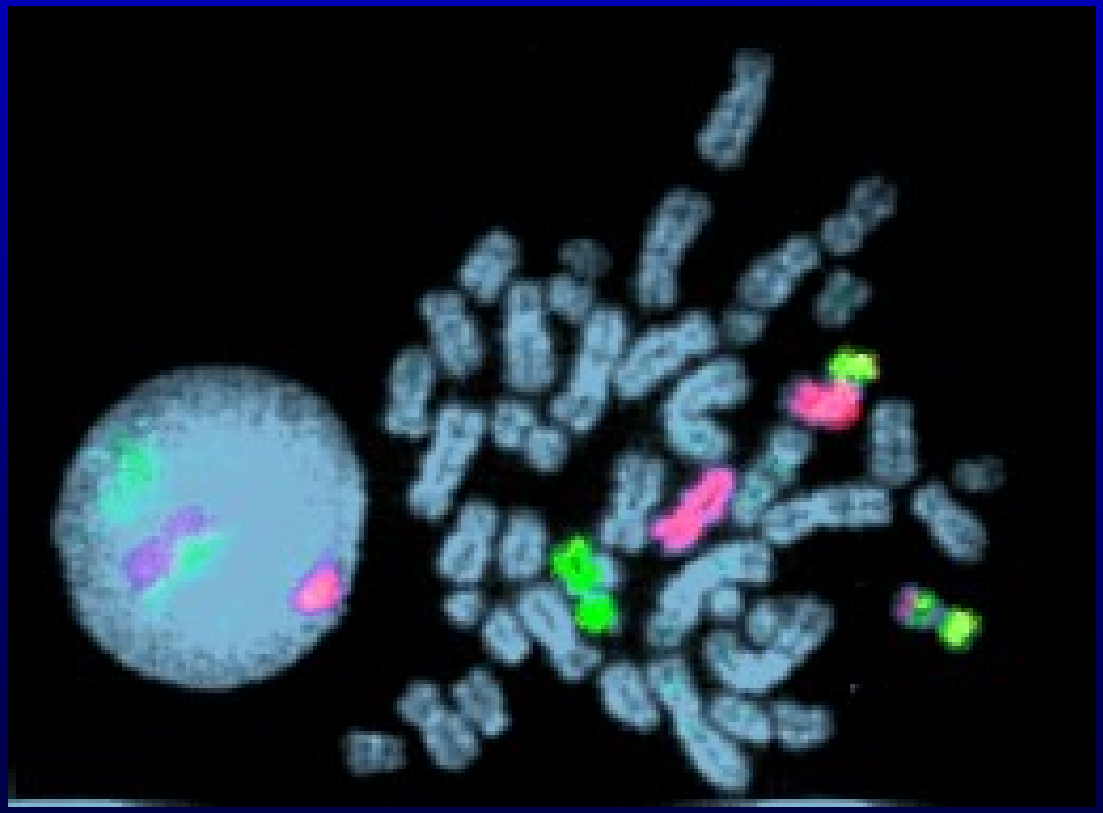


Formation of a reciprocal translocation

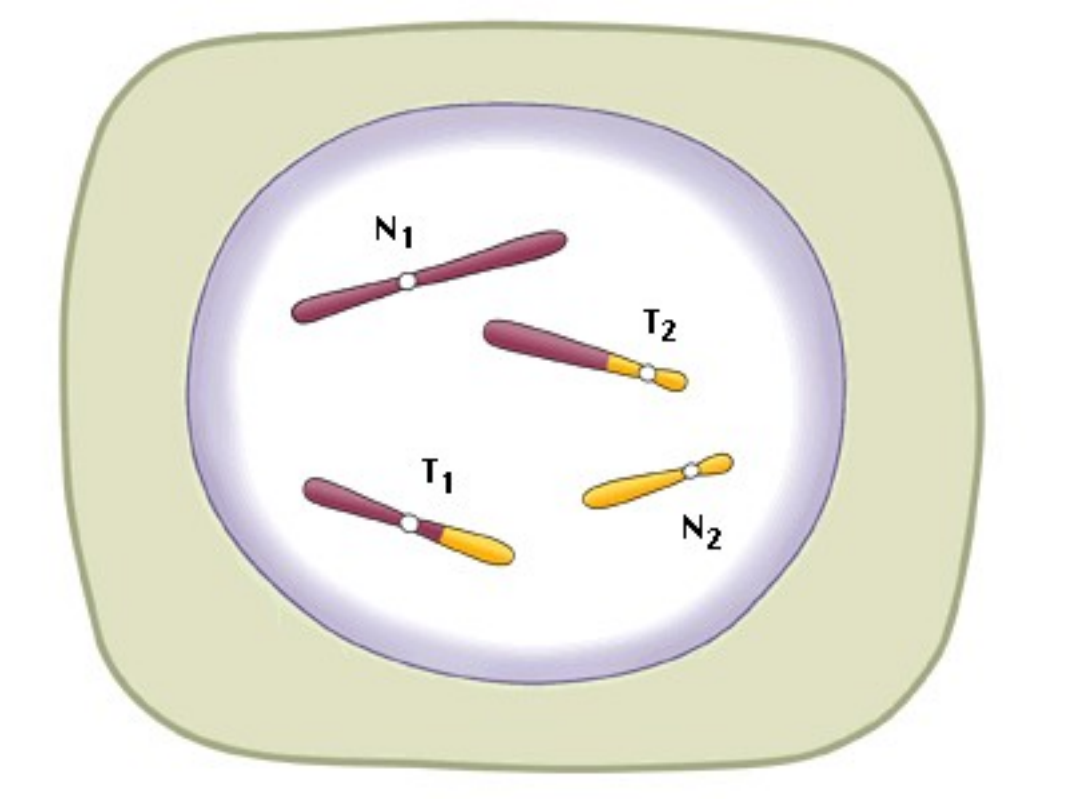


10:11 translocation

Chromosome painting can be used to detect translocations



Translocation at meiosis

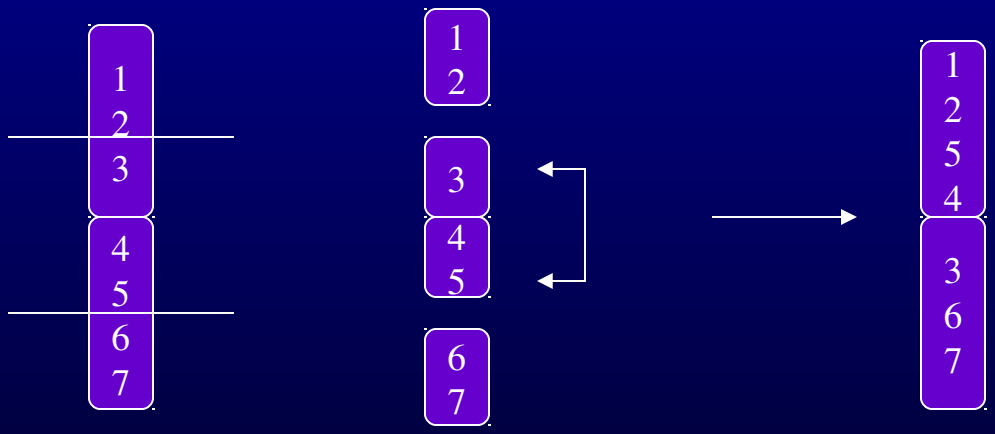


How do balanced translocations cause infertility?

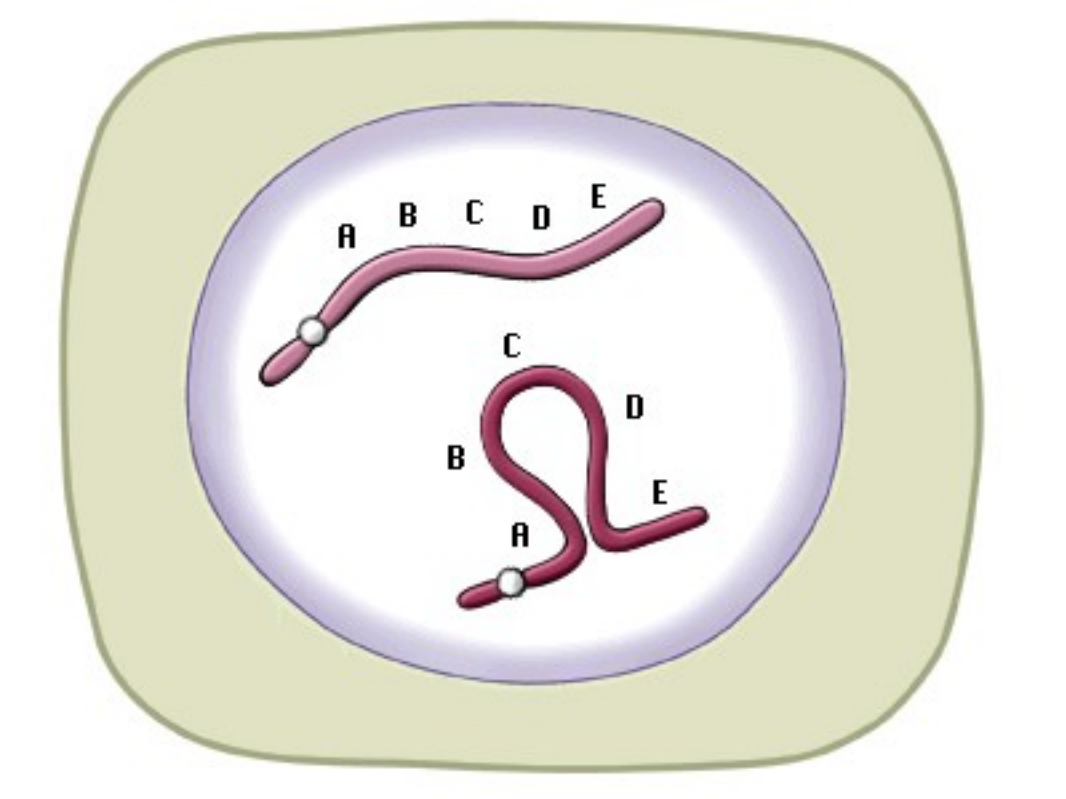
- Messing up of meiosis
- Reduced recombination in pairing cross
- Production of unbalanced gametes leading to severely affected embryos that may not develop

Structural abnormalities cont.

- Inversions:
  - Two breakpoints -> Piece inverts
  - Usually no clinical features unless gene disrupted
  - Can lead to reduced fertility
  - Paracentric or Pericentric



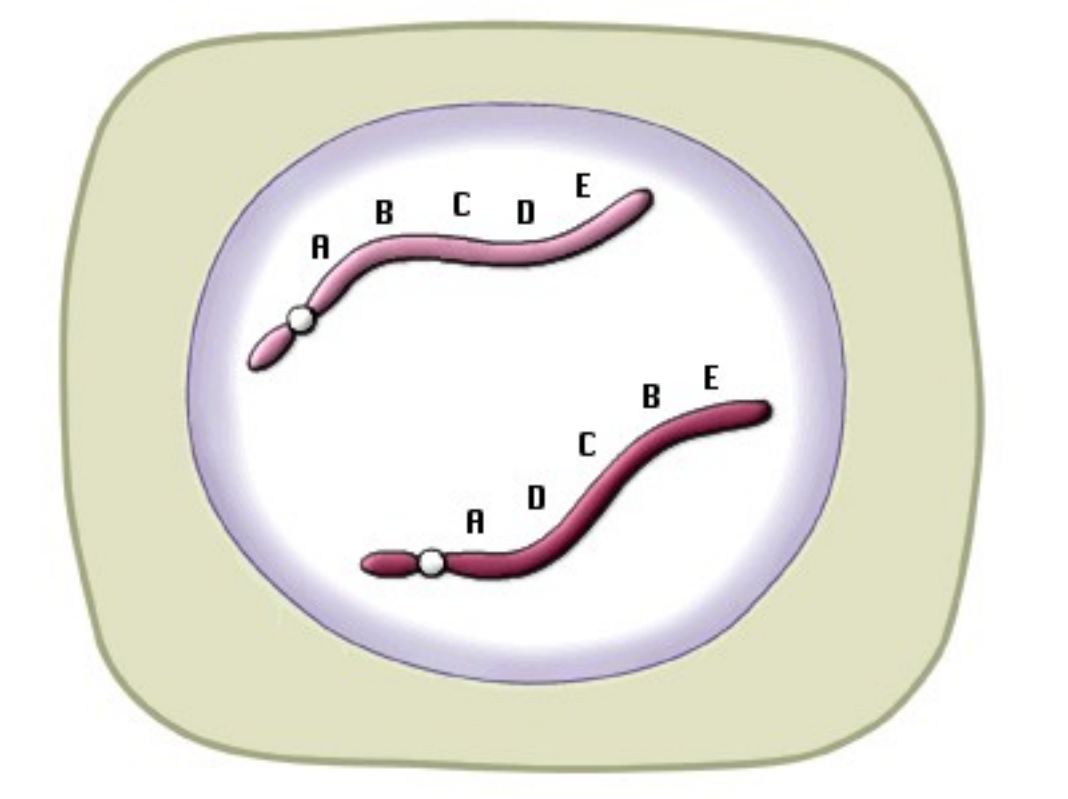
Formation of an inversion



How do inversions cause infertility?

- Messing up of meiosis
- Reduced genetic recombination within pairing loop
- Production of unbalanced gametes leading to severely affected embryos that may not develop

Inversion at meiosis



Further structural abnormalities

- Ring chromosomes: Deletion of both arms, severe clinical features
- Marker chromosomes: Unidentifiable chromosome
- Isochromosomes: Chromosome with 2 p-arms or 2-q-arms. (mirror image).
- Dicentrics: Chromosomes with 2 centromeres (primary constrictions, can be isochromosomes)
- Breaks, gaps and fragile sites
  - Chromatid or chromosome
  - Gaps are aligned, breaks are not
  - Common points of breakage -> Fragile sites
    - fragile X (mild mental retardation syndrome)
    - Others not clinically significant
    - Some syndromes particularly prone to chromosome breaks, esp. if put under stressful culture conditions
- Complex rearrangements: rare cases of combinations of any type of structural abnormality

Chromosomes, Disease and Gene Mapping

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Chromosomes and Infertility - further issues

- Aneuploidies XXY, XYY, XO
- Inversions
- Balanced translocations
- Y chromosome deletions
- Maternal age effect for trisomy
- Aneuploidy in the sperm

Chromosomes and Infertility - further issues

- Aneuploidies XXY, XYY, XO
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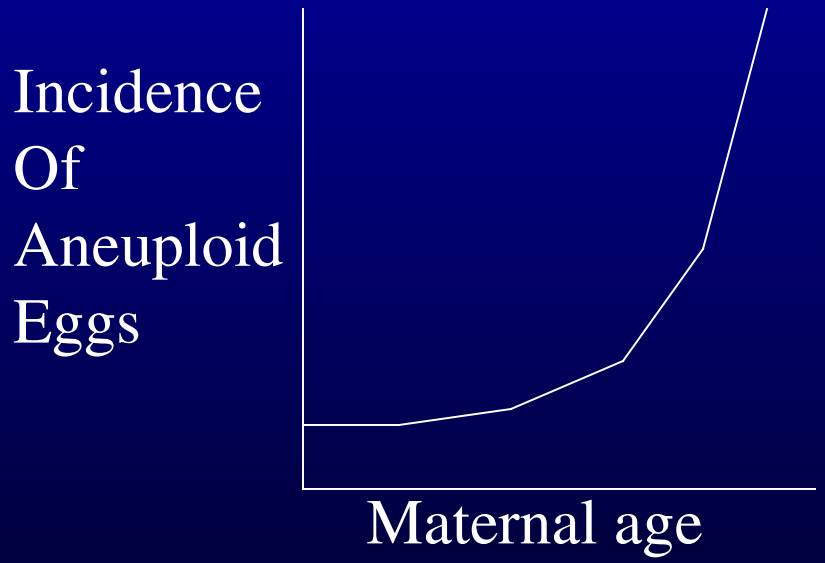
- Aneuploidy in the sperm

Maternal age effect

- Women are more likely to produce Down Syndrome children as they get older
- Also more likely to be infertile because of aneuploid eggs



Chromosomes and Infertility

- Aneuploidies XXY, XYY, XO
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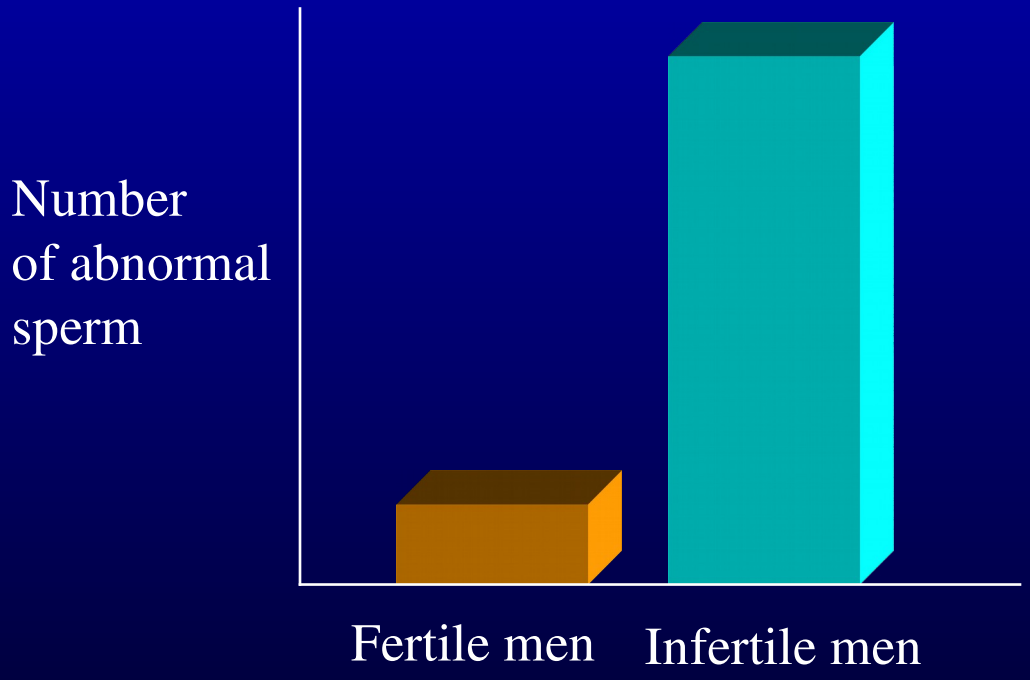
- Aneuploidy in the sperm

Sperm aneuploidy - Background

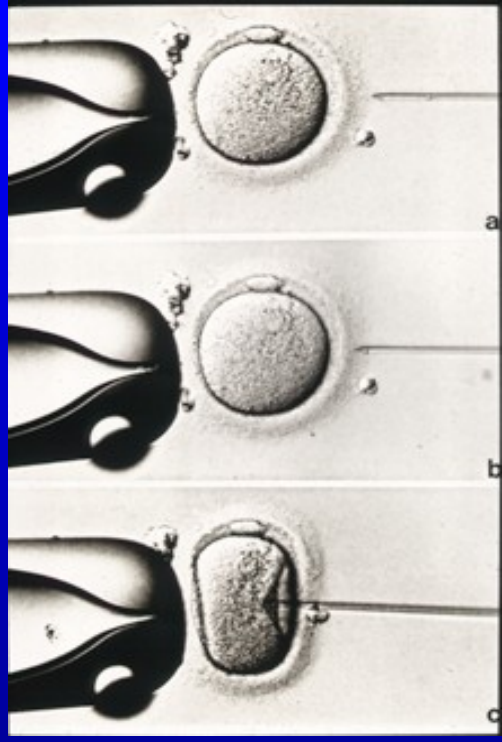
- Definition
  - The proportion of sperm in an ejaculate with an extra chromosome
- Previous studies shown effects of age and factors such as smoking that increase the incidence of sperm aneuploidy
- Several authors have reported a dramatic association with severe infertility

Studies of Sperm

- All men have a proportion of sperm that are abnormal
- Infertile men have more than most



Studies of Infertility and sperm aneuploidy



- Infertile men should not procreate
- BUT male infertility is treated by taking what few sperm there are and directly injecting into an egg (ICSI)
- Is this treatment more likely to give rise to babies with genetic abnormalities?

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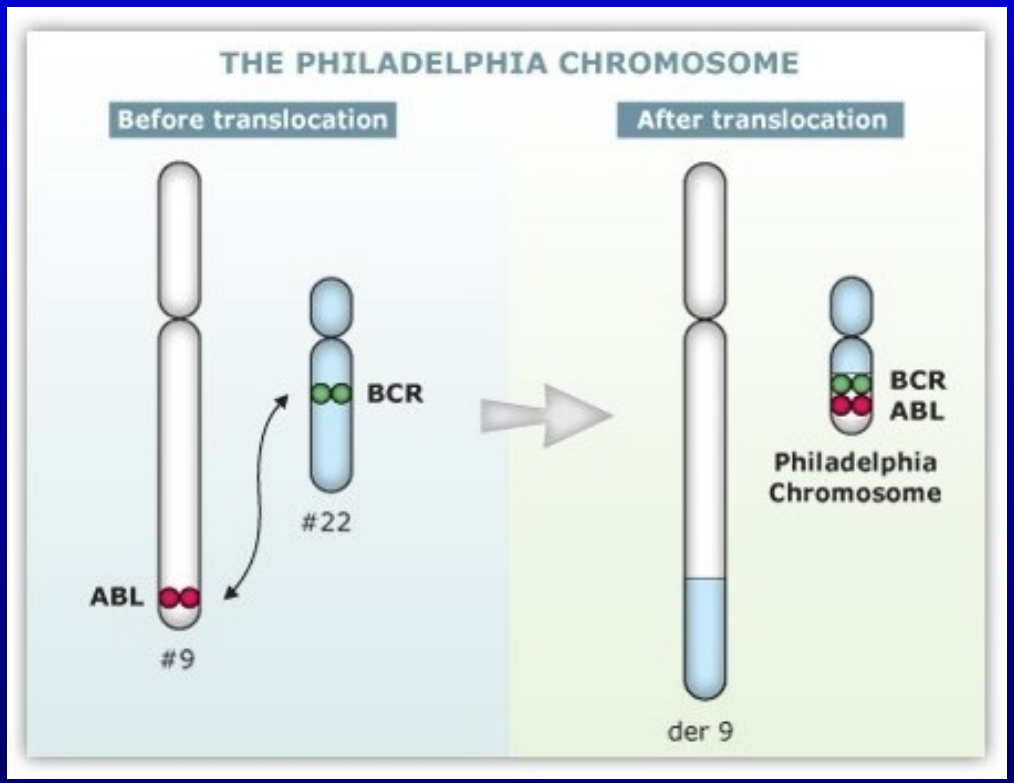
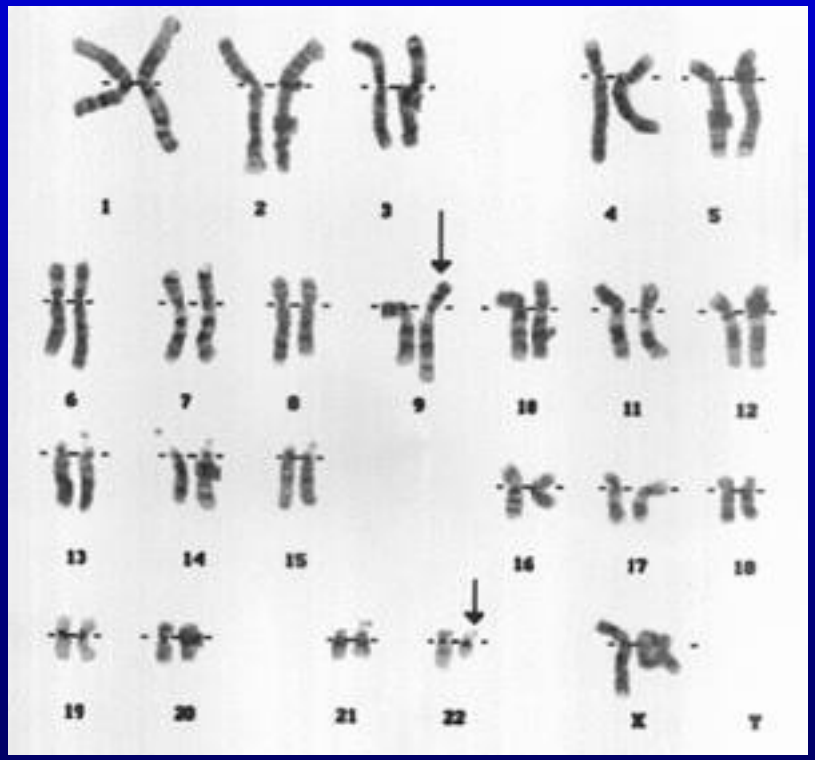
Chromosomes and cancer

- Genetic studies in human tumours basically look for two types of gene
  - Tumour supressor genes (TSGs)
  - Oncogenes
- Studying the tumour alone (not the constitutional karyotype of the individual)
  - Consistent deletions can indicate TSGs
  - Amplifications, aneuploidy can indicate oncogenes
  - Consistent translocations can also indicate aneuploidy

Philadelphia chromosome





Methods of Study

- G banding
  - Oncogenes also indicated by homogeneously staining regions and double minutes
- FISH
  - Picking out individual chromosome translocations
  - Interphase cytogenetics (esp solid tumours)
- Comparative genomic hybridization (CGH)
  - Chromosomal
  - Microarray

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Cytogenetic investigations are expensive

- It is inappropriate to cytogenetically screen individuals at random
- Resources need to be directed
- Does the patient have a chromosome anomaly relevant to his or her clinical problem?

Major referral categories

- 4-12 weeks gestation (1st trimester)
- 12 weeks to term (2nd and 3rd trimesters)
- Neonatal period
- Early childhood
- Puberty and sexual development
- Problems in fertility and reproductive failure
- Adulthood

4-12 weeks' gestation

- Spontaneous abortions
- Most chromosomally abnormal conceptions lost here
- Any trisomy
- 45,X
- Unbalanced rearrangements (large gains or losses - partial trisomy/monosomy)
- Triploids

12 weeks to term

- Abnormalities picked up on ultrasound
- Trisomies 13, 18, 21
- Unbalanced Robertsonian translocations producing trisomy
- 45,X
- Some triploids
- Some unbalanced translocations
- Offered prenatal diagnosis (see later)

Neonatal period

- Child with multiple congenital abnormalities
- Trisomies 13,18, 21 (including mosaics)
- Unbalanced Robertsonian translocations
- Deletions esp. 4p, 5p, 9p, 13q, 18p, 18q
- Small unbalanced inherited structural rearrangements
- Rings esp. 4, 5, 13, 18

Early development

- Missed by paediatricians in neonatal period but fail to achieve mental and physical milestones
- Subtle chromosome abnormalities e.g. small rearrangements/deletions, marker chromosomes, fragile X, *apparently balanced* rearrangements, some mosaic trisomies

Puberty and secondary sexual development

- Inappropriate sexual development
- 45,X
- Deleted and rearranged X (including ring X)
- 46,XY females
- 46,XX males
- 47,XXY (Klinefelter syndrome)

Infertility and reproductive failure

- Patients present at an infertility clinic
- All aforementioned sex chromosome abnormalities
- XYY
- Balanced structural rearrangements
- Marker chromosomes
- X or Y; autosome translocations
- Y structural rearrangements
- Y deletions
- Aneuploidy in the sperm

Other adult referrals

- Often institutionalised patients
- Often patient as part of a larger family study
- Often fragile X

Major referral categories

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- Copy number variation (CNV)

Why? -- Reasons for prenatal diagnosis

Invasive sampling procedure small but significant risk to the fetus

sampling procedure and lab analysis expensive so resources channelled towards high risk group

Cytogenetic abnormalities

- Older mothers (risk of trisomy)
- Families with previous trisomic pregnancies or strong family history of trisomy
- Couples one of whom has a balanced rearrangement

Cytogenetic abnormalities

- Cases where an abnormality is suggested on ultrasound (see previous)
- Abnormal serum screen
  - Screen of maternal serum levels for alpha fetal protein, human chorionic gonadotrophin (HCG), oestriol, this coupled with maternal age can give a risk assessment for likelihood of trisomy, detects 66% of trisomy 21.
  - Screen at 16 weeks

How?

- Amniocentesis (14-16 weeks) mostly abnormal serum screen and advanced maternal age
- Chorionic villus sample (9-13 weeks)
- Fetal blood (later) if the above give spurious results and late referrals

Problems

- Mosaicism
  - Some normal and some abnormal cells in conceptus
- Contamination of maternal cells

What next?

- Families are offered the choice of a therapeutic abortion
- Sometimes they just want to know the result so that they can prepare for the affected child
- Very dependent on the family involved and on the severity of the disorder

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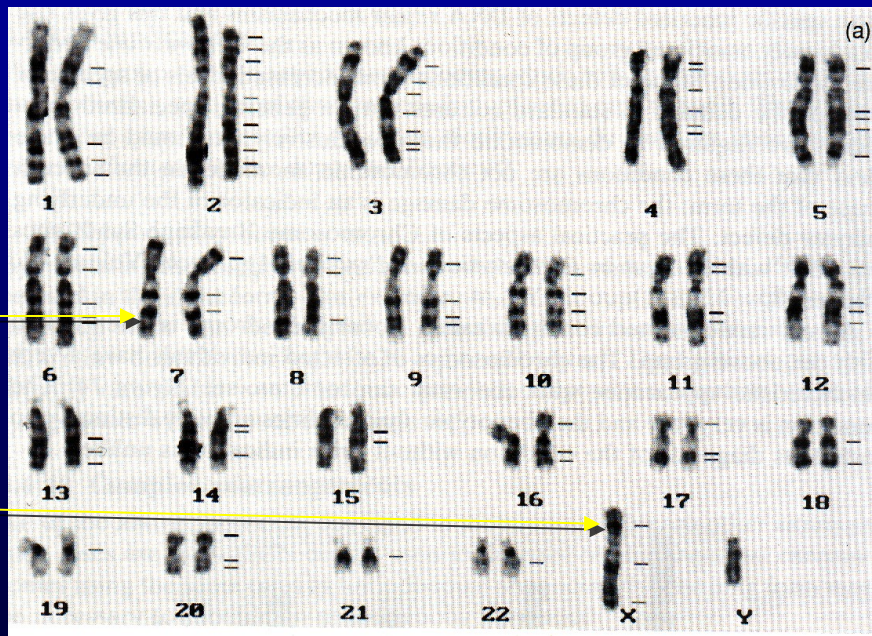
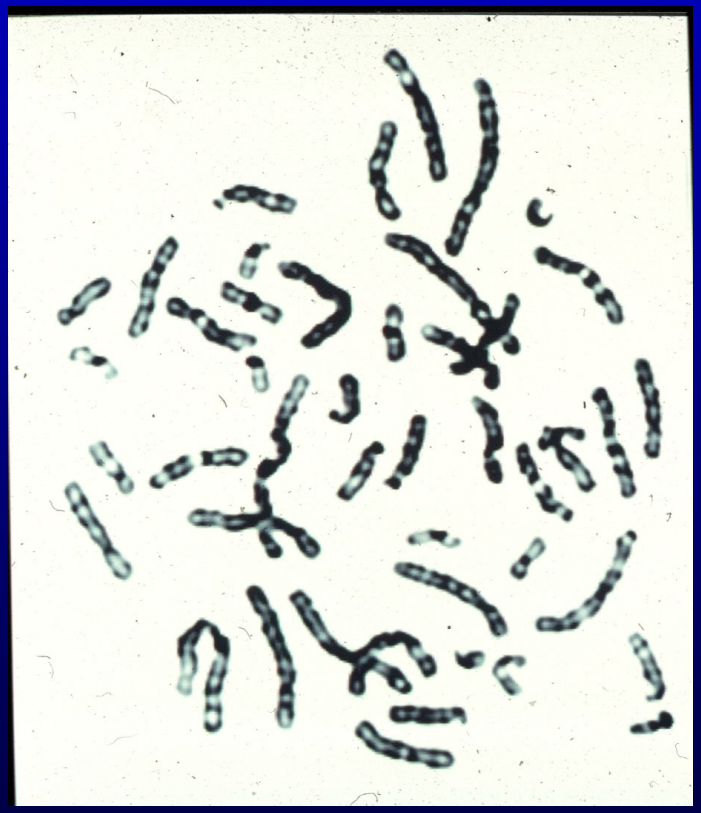


Cystic fibrosis gene

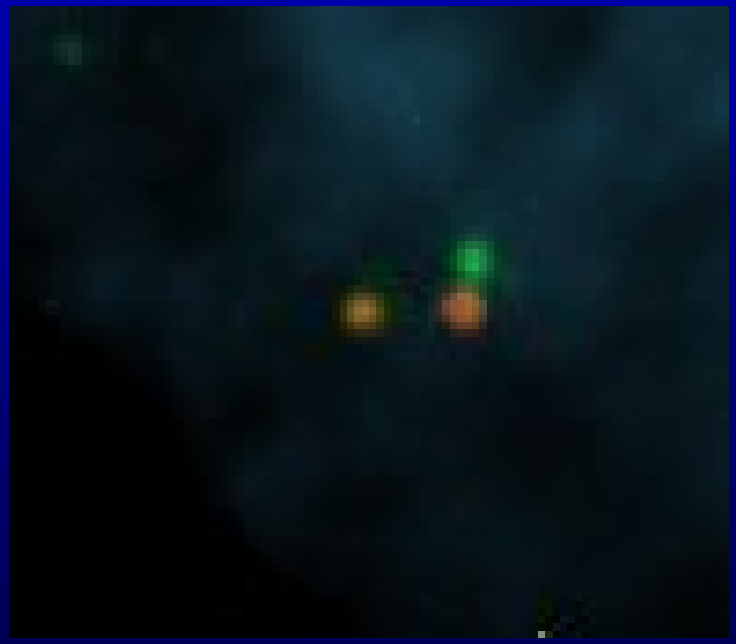
Muscular dystrophy gene

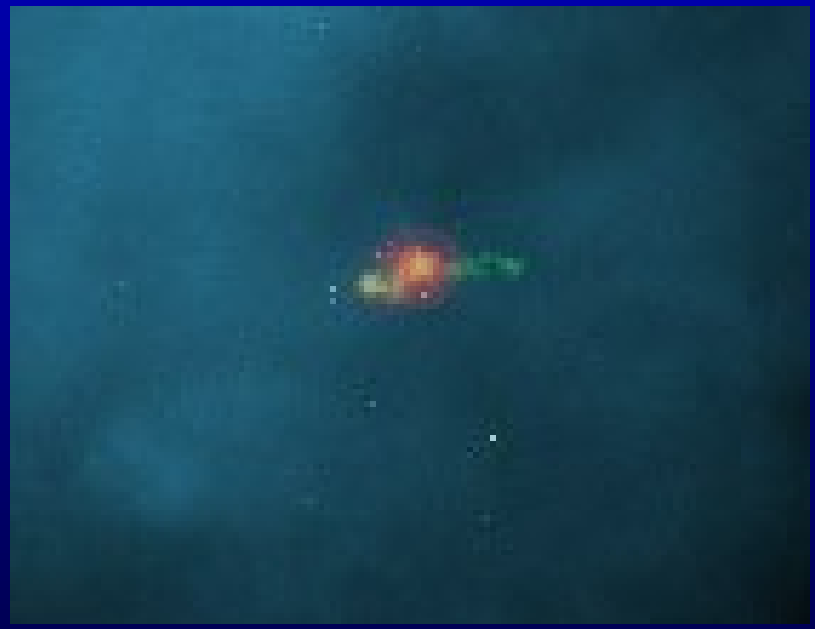
FISH for Gene mapping on chromosomes



FISH for gene mapping in nuclei

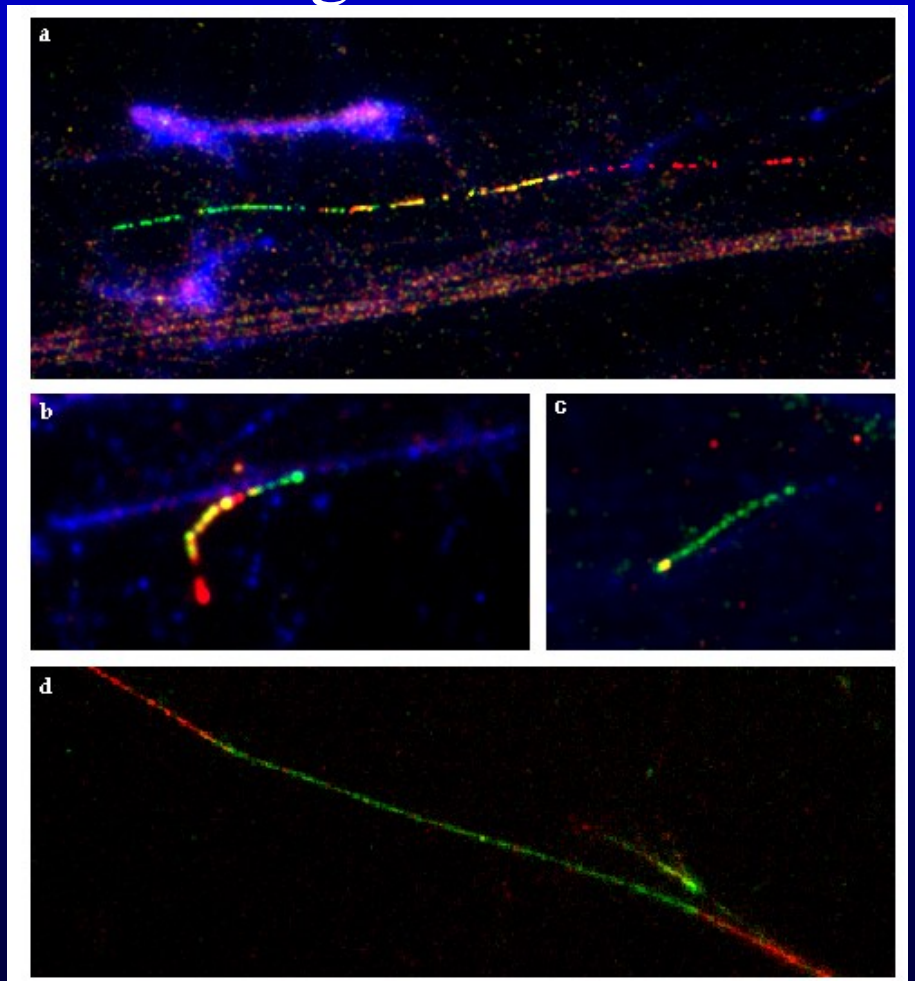
- Gives much better resolution





Fibre FISH

- Stretching out DNA gives even better resolution



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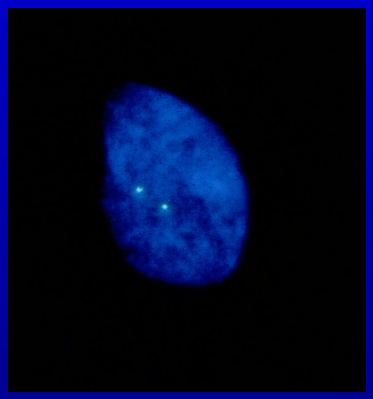
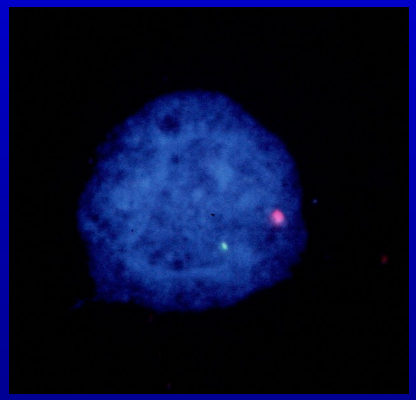
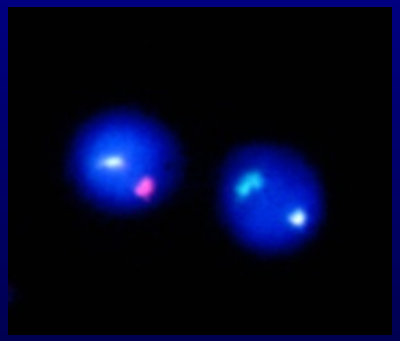
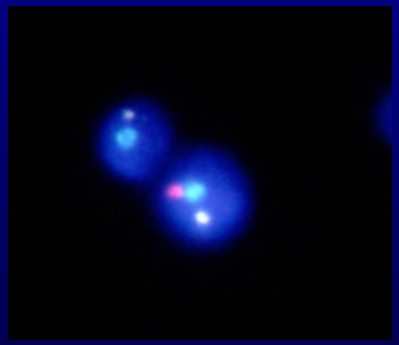
Counting chromosomes in non-dividing nuclei





- Sex chromosomes in single cells from embryos

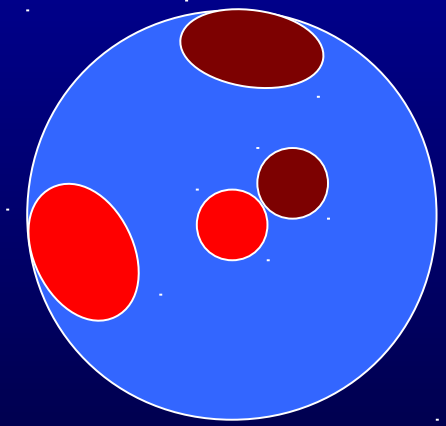


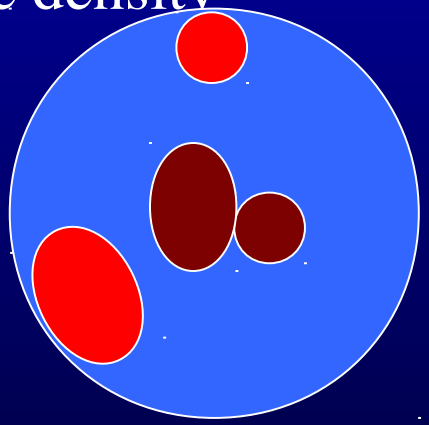


- Sex chromosomes in sperm

Nuclear (Genome) organisation

- Nuclear organisation – positions of chromosomes in the interphase nucleus
- Implicated in:
  - Disease
  - Development
- Two models - chromosome size / gene density





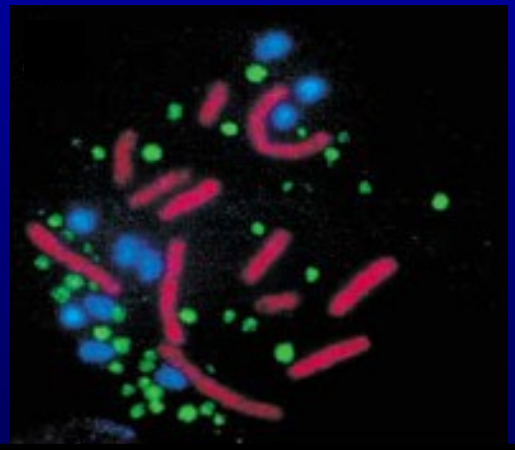
Nuclear organisation in chicken

- Big chromosomes on the periphery
- Smaller chromosomes at the inside
- Which is fine except that the smaller chromosomes are more gene dense

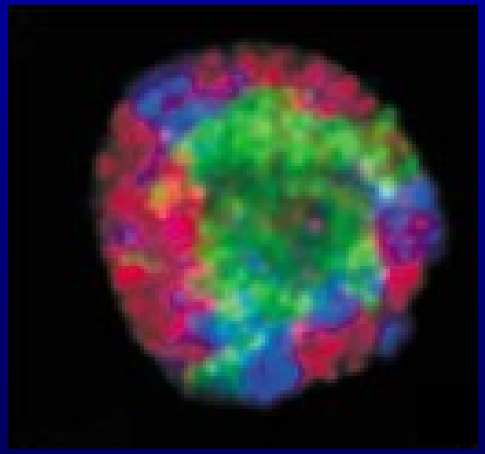
1-5, Z

6-10

Micros



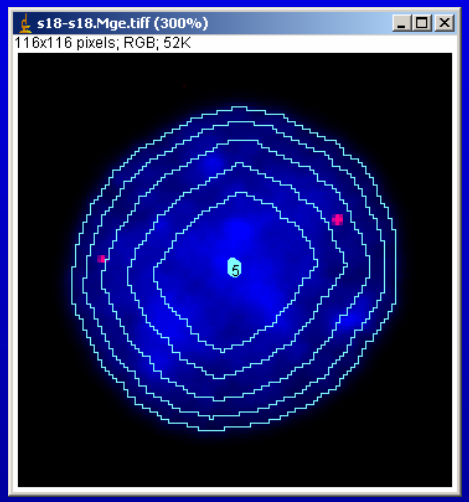
From Habermann et al, 2001



Other models

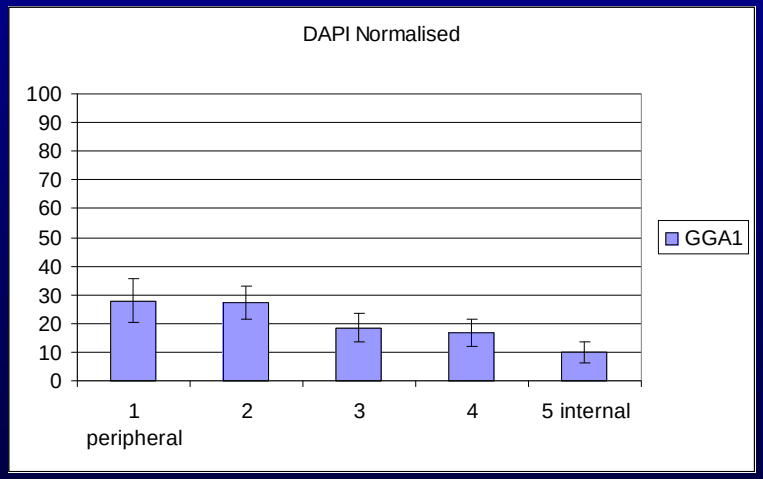
- Random
  - No discernable pattern
- Chromo-centric
  - Centromeres in the centre
  - Sperm
  - Many mouse cells
- Telo-centric
  - Telomeres in the centre
  - Cells of the eye
  - Nuclei smaller, allows more light through

Methodology



Chromosome position

- Automated ring template
- Measure the relative position of the signal
- Measure 50 or so cells
- Adjust for relative DNA content



Alterations associated with disease/development

- X-inactivation
  - Second X chromosome at nuclear periphery
- Senescent and quiescent cells
  - Tendency to randomness
- Reproduction
  - Sex chromosomes migrate to centre during spermatogenesis
  - Sex chromosomes more random in in fertile males
- Cancer
  - Movement of chromosome 18 to nuclear centre
- Epilepsy
  - Brain cortex cells
  - Small movements in chromosomes 1, 9, Y
- Nuclear lamin related diseases
  - Emery-Dreifuss Muscular Dystrophy

Individual genes

- Active genes sit more towards edge of chromosome territory
  - Access transcriptional machinery
- More active genes near foci of DNA polymerase II (again transcriptional machinery)
- Less active genes cluster in heterochromatic regions of nucleus
- Genes move more towards nuclear centre when activated

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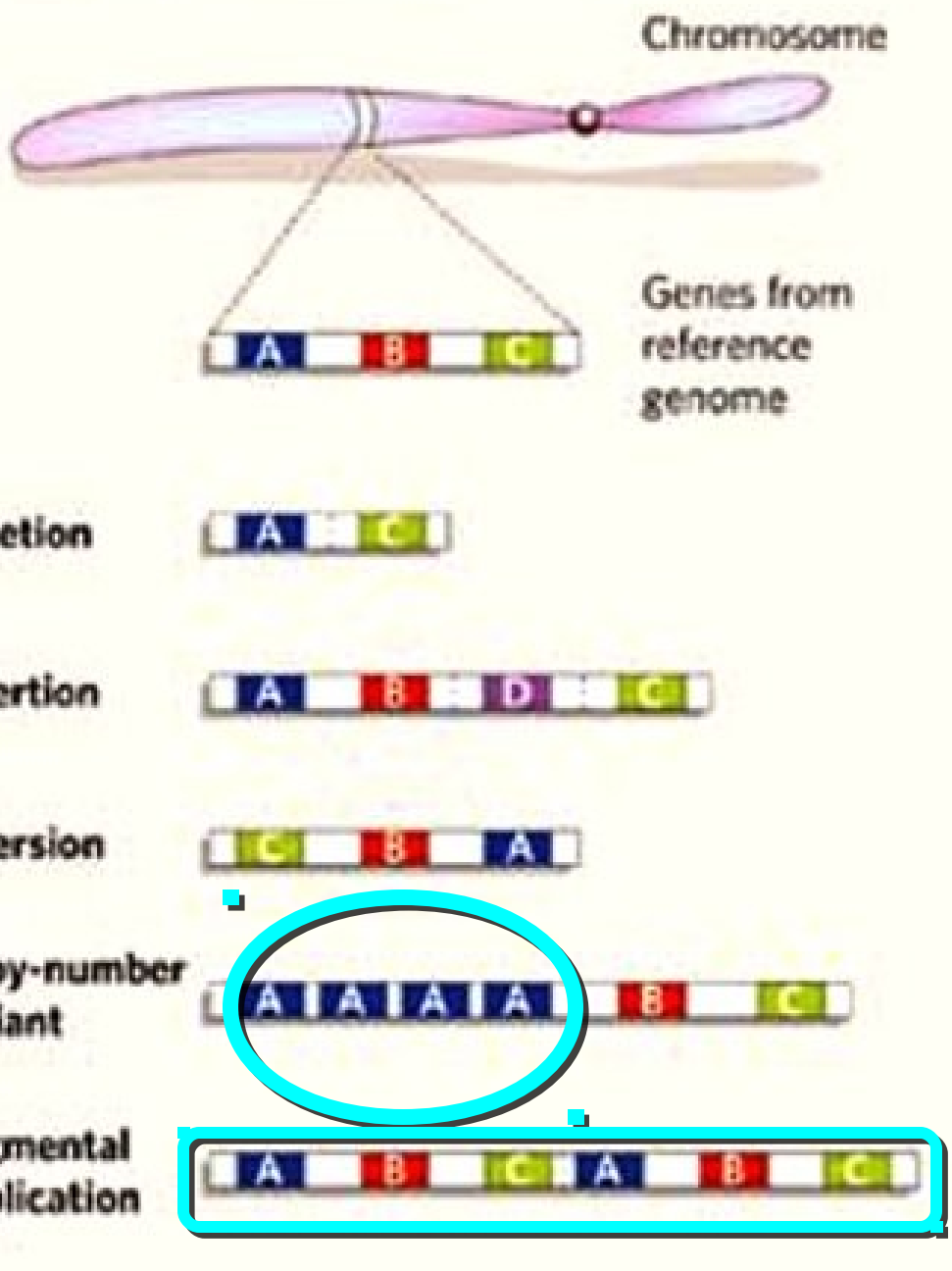
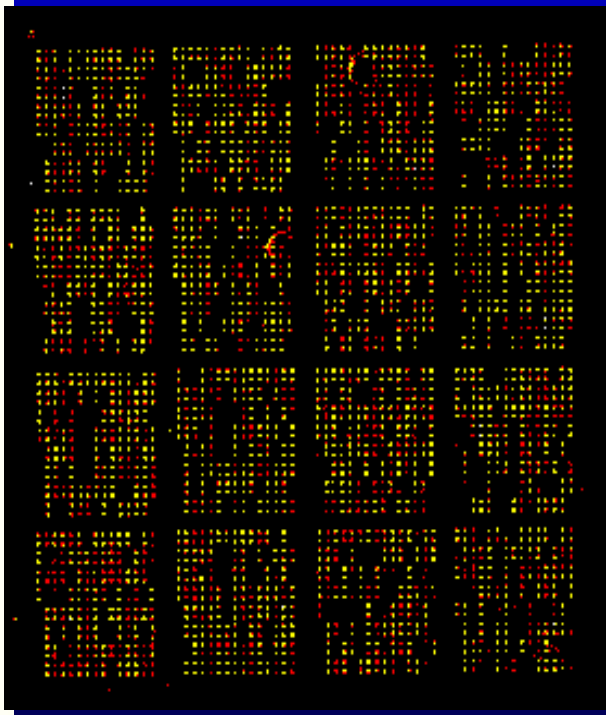
VARIATIONS IN OUR GENOMES





Copy Number Variation

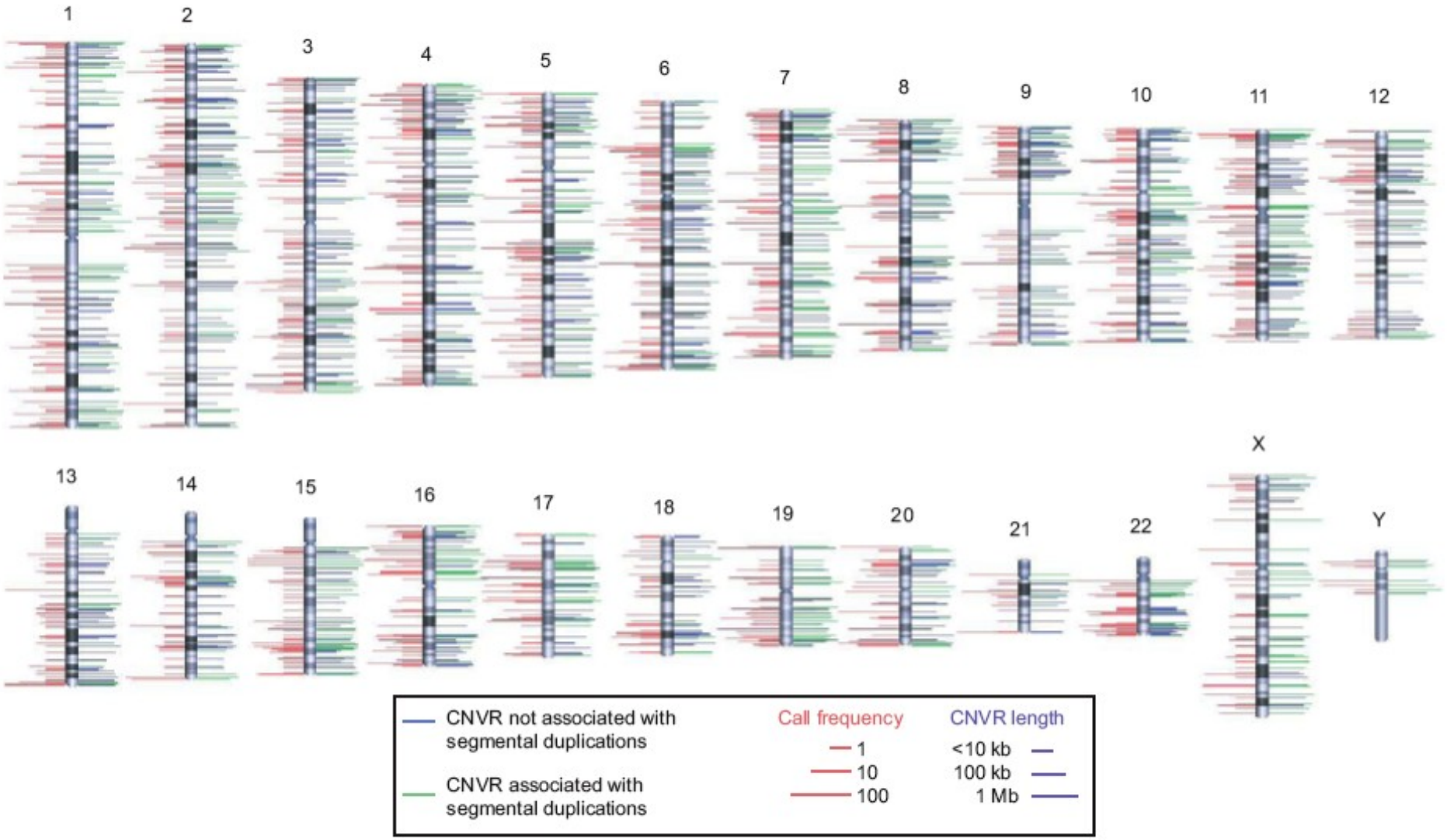
- DNA segment 1kb or larger and present at variable copy number in comparison with a reference genome
  - Not including insertions, deletions or transposable elements
  - Range from 1kb to several Mb
- Segmental duplications
  - Large, low copy CNVs
  - Substrates for non-homologous recombination
- Functionally significance yet to be fully ascertained
  - Variation between individuals
    - Linked to phenotype/disease?
  - Variation between strains
    - Linked to characteristics?
  - Variation between species
    - Linked to evolution?

Human Genome CNV Studies

- International DNA and cell line collection derived from apparently healthy individuals
  - 30 parent offspring trios Yoruba Nigeria (YRI)
  - 30 parent offspring trios European descent Utah (CEU)
  - 45 unrelated Japanese Tokyo (JPT)
  - 45 unrelated Han Chinese Beijing (CHB)

Redon et al., 2006 Nature 444;444

Human Genome CNV Studies



CNVs in Humans

- 1447 discrete CNVs
  - 12% of genome
  - 360Mb *(*compared to 3Mb bi-allelic SNPs)
- Relevance not yet fully understood
  - But it really is flooding the literature
- Disrupt genes and alter gene dosage
  - Gene expression
  - Phenotypic variation
  - Adaptation and evolution
- Cause or confer risk to disease
  - Linked specific genetic disease pathologies
    - The trick is to differentiate the normal variants from the disease related ones
  - Evidence for effects on complex traits
  - Pre-dispose to deleterious genetic changes
  - Basis for variation to drug response?

Medical Relevance

- CNVs and syndromes within regions commonly deleted
  - Di-George
  - Prader-Willi/Angelman
  - Smith-Magenis
  - Williams-Beuren
- Causative alleles at genes strongly associated with specific diseases e.g.
  - Parkinson's,
  - Alzheimer's
  - Spinal muscular atrophy
  - Schizophrenia
- From ESHG:
  - Overgrowth syndromes
  - Congenital heart defects
  - Mental retardation
  - Multiple congenital abnormalities
  - Cancer

Chromosomes, Disease and Gene Mapping - SUMMARY

- What are chromosomes? - COILED BODIES OF DNA/PROTEIN AT CELL DIV.
- How do we make chromosomes? - DIVIDING, ARREST, SWELL, FIX, STAIN
  - Samples - BLOOD, SKIN, CVS, AMNIOCENTESIS
  - G-banding - TRYPSIN, GIEMSA
  - FISH - FLUORESCENT, MANY APPLICATIONS
- How to we analyse chromosomes? - COUNT, SIZE, CENTROMERE, BANDING
- Chromosomes and disease
  - Numerical abnormalities - TRISOMY, MONOSOMY, TRIPLOID
  - Structural abnormalities - DELETIONS, TRANSLOCATIONS, INVERSIONS ETC.
  - Fertility issues - MATERNAL AGE EFFECT, SPERM ANEUPLOIDY
- Diagnosis and genetic counselling
  - Referral categories - FROM 0-12 WEEKS TO ADULT
  - Prenatal diagnosis (why, how, what next?) - EXPENSIVE, CVS, ?ABORT
- Chromosomes and gene mapping - METAPHASE AND INTERPHASE
- Nuclear organisation, development and disease - TERRITORIES AND GENES
- Copy number variation (CNV) - MICROARRAYS, NEW ERA CYTOGENETICS